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HOWARD EVERETT PARSON

1897–1943

H. L. Crane

Howard Everett Parson was born at Smith's Creek, Michigan, on May 13, 1897. He was the son of a farmer and grew up on the farm where he learned about fruits and the diseases attacking them. He attended the Beech Grade School, Wales Township, St. Clair County, Michigan. After completing his Port Huron High School training, he spent a year at Michigan State Normal School at Ypsilanti, Michigan, his schooling there being interrupted to enter the United States Army in World War I, at Ann Arbor, Michigan. He served in a Medical Company of a Casualty Detachment in England and France for approximately one year, being honorably discharged at Camp Custer, Michigan, March 1, 1919.

Upon his return to civilian life, Mr. Parson entered Michigan State Agricultural College and received his Bachelor of Science Degree in 1923. After receiving his Bachelor's Degree from Michigan State College, he was employed as Field Assistant with the Bureau of Plant Industry during the summer months of 1922 and 1924 and served in the position as Agent in the same Bureau, a portion of the year 1927. During school year, 1924–1925, he taught agriculture in Smith-Hughes School at Mesick, Michigan. This experience led him to decide to obtain additional training so that he might go into research work. In 1925 he enrolled in the Graduate School at the University of Minnesota and in 1928 obtained the Master of Science Degree from that University. His graduate work dealt with physiologic specialization in *Puccinia coronata avenae*. Part of this time he was Instructor in the School of Agriculture and Research Assistant in the Minnesota Agricultural Experiment Station. In 1927 he worked on the epidemiology of cereal rusts for the United States Department of Agriculture.

He was appointed to the position of Junior Pathologist in the Bureau of Plant Industry for work on pecan diseases, with headquarters at Thomasville, Georgia, on January 2, 1929. In this capacity, he did very important work in Georgia and Alabama, later being transferred to Shreveport, Louisiana, where he continued his investigations, working primarily on pecan leaf-spot diseases and their control, as well as on a virus disease known as bunch disease which affected the pecan and water hickory trees of that area and surrounding areas in Texas, Arkansas, and Mississippi.

He married Adelaide Blanche Bunnell on October 6, 1930, and they have one daughter.

Mr. Parson died November 18, 1943, in Shreveport, Louisiana.

He was author or co-author of the following papers:

Physiologic specialization in Puccinia coronata evenae. Phytopath. **17**: 783–790. 1927. (With J. G. Leach and H. W. Johnson.) The use of acidulated mercuric chloride in disinfecting potato tubers for the control of Rhizoctonia. Phytopath. **19**: 713–724. 1929. (With G. F. Moznette, C. B. Nickels, W. C. Pierce, T. L. Bissell, J. B. Demaree, J. R. Cole, and John R. Large.) Insects and diseases of the pecan and their control. U. S. Dept. Agr., Farmers' Bull. 1829. 1940.

HOWARD EVERETT PARSON
1897–1943

ON POD SPOTS IN PEPPERS

H. Bremer

(Accepted for publication September 1, 1944)

In November, 1937, the majority of the pods in a field of peppers inspected at Bornova, near Izmir (Smyrn), Turkey, were injured by rotted spots with a layer of shining black mold on them. Microscopic examination proved the fungus to be a species of *Alternaria*. Pure cultures were produced on agar, one of which was identified by the Centraalbureau voor Schimmelcultures at Baarn (Holland) as *Alternaria longipes* (Ell. et Ev.) Tisd. et Wadk.

This species of *Alternaria* is known to be a parasite of tobacco. Tobacco and pepper leaves were inoculated with an isolate from pepper, with negative results. Pepper fruits and stems became infected, if they had been wounded before inoculation. Thus, the fungus is a wound parasite, but it cannot be the primary cause of the spots.

In 1938, spots of the same shape but without the mold layer (Fig. 1) were frequent on peppers in the same region. They appeared in July and August, in the hottest season, on the sides or the tops of the full-grown semimature or mature pods.

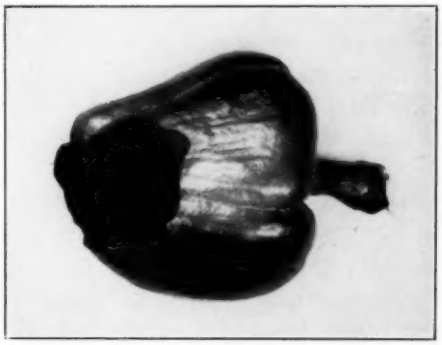

Fig. 1. Pod spot of pepper.

At first, lesions are bright-colored, somewhat sunken. In dry weather the dead skin of the spots becomes parchment-like, hard and dry; if damp weather prevails, the mold layer mentioned may develop on it. The spots often become very large, sometimes extending over almost the whole pod making it in every case unmarketable.

Pod spot is not unknown. In Florida, similar symptoms characterize "sunscald" or "blossom-end rot,"[1] in Hungary "Dorrfleckenkrankheit (dry spot disease)."[2] In Florida the damage in some years reaches 50 per cent.

[1] Weber, G. F. Diseases of peppers in Florida. Fla. Agr. Exp. Sta. Bul. 244. 1932.
[2] Szirmai, J. Die "Dorrfleckenkrankheit" (Hitzeschaden) des Paprikas. Phytopath. Zeitschrift **11**: 1–13. 1938.

In Hungary 4 to 12 per cent is said to be the average loss. According to G. F. Weber, the Florida author, the possible causes are either the destroying effects of direct sun rays (sunscald) or insufficient or fluctuating water supply (blossom-end rot). This author says that the two diseases are likely to be confounded with each other but can be distinguished by the position of the spots. In sunscald the spots are on the sides of pods, while blossom-end rot appears on the top (blossom end) of the fruits. Szirmai, who studied the disease in Hungary, believes that the spots are not caused by radiation but by heat. He was able to reproduce similar spots by hot air of 50–52° C., provided that the fruits were moistened with water. Now these conditions seem rather abnormal; in direct sunlight the temperature sometimes may be 50–52° C., but moisture is not likely to be present, at least in arid regions. The results obtained by Szirmai are not to be doubted, but we cannot believe that the pod spots always are brought about in the same manner in nature.

Pod spot occurs in many pepper fields in Turkey, and sometimes up to 30 per cent and more of the fruits are made useless by the spots.

Except for secondary contaminations, fungi or bacteria could not be found in the diseased fruit tissue nor isolated from it. Therefore, the disease should be considered a physiological one, caused by adverse conditions.

The distinctions between sunscald and blossom-end rot, as given by Weber, did not always hold. In most cases the spots were lateral, often extending to the top of the pod; sometimes they were limited to the top. All types occurred in one field and close to each other, thus indicating the same origin. The symptoms of pod spot generally resemble somewhat those of blossom-end rot of tomatoes. In closely related plants, such as the solanaceous species, pepper and tomato, every local breakdown of the fruit-wall tissue may result in similar symptoms. In a few cases spots of the same appearance have been found on the tops of fruits of egg plant (*Solanum melongena* L.), another related species. On the other hand, it is possible that the same cause may result in different localization of spots in pepper and tomato, since their fruits are constructed differently in spite of the relationship of the two species. Pod spots in the native variety "Domates biberi" (tomato pepper), the fruits of which resemble somewhat tomatoes in their short and compact shape, were particularly similar to spots of blossom-end rot in tomatoes.

Thus, we believe that it is not possible to decide, by the external appearance of the diseased fruits, whether sunscald (heat damage) is present or blossom-end rot (the result of disturbed water balance).

No doubt the sun plays an important rôle in the origin of the damage. The injury appears at its highest degree in midsummer. In the hot days of August, 1940, many of the fruits were damaged in the experimental field of the Central Institute of Plant Protection at Ankara. All the pods that developed later remained free from disease. We tried, in the hottest days, to reproduce the disease experimentally by moistening fruits in full sunlight at noon, according to the work of Szirmai, but the results were negative.

Localization of the spots towards the sun, however, strengthens the assumption of an effect of sunrays. In most cases, as has already been observed by Szirmai, spots are on the south side of the pod. But of 28 spotted fruits in a small experimental plot (Ankara, 1940), for instance, 3 were spotted on the east side, 19 on the south side, 6 on the west, and none on the north side. These observations seem to indicate "sunscald." However, the effect of the sun cannot be such a simple one. Weber, too, supposes that other factors must join the radiation of the sun in order to produce the disease: "Sunscald is also common on varieties that show poor, little or no growth." Furthermore, "some possible contributing factors in respect to these areas are . . . sections protected by woods and hillsides" and "lighter, more thirsty soils."

WATER BALANCE IN PEPPER

The similarity of conditions accompanying sunscald and blossom-end rot lead us to question the effect of disturbed water balance. In 1940, at the time that pepper-pod spot was severe in the experimental field at Ankara, a heavy attack of blossom-end rot occurred simultaneously in a neighboring field of tomatoes. This raises the question as to whether there are signs of a disturbed water balance in the diseased pepper plants. To approach the solution of this question, the velocity of wilting in leaves of pepper was measured. Usually three leaves of similar area and level of insertion were cut from a pepper plant with many spotted pods and three corresponding leaves from a neighboring plant with healthy pods. To have a starting point as uniform as possible, the leaves were put in a moist chamber for half an hour, then weighed, laid out in the laboratory for drying and weighed again after definite periods. In 1938, plants were taken at random from the same variety each time, from the experimental field of Bornova. In 1939, the plants taken for the experiments were all descendants of a single plant in each variety. These experiments have been repeated nine times, and have always given a uniform result: leaves of plants with many spotted pods wilted and lost weight more slowly than leaves of plants with healthy pods. For instance, in the five experiments with descendants of single plants, the percentage losses in weight were as in table 1.

For technical reasons, it was not possible to make the readings at the same

TABLE 1.—*Loss of fresh weight in wilting leaves of pepper*

Plants with healthy pods		Plants with spotted pods	
Duration of wilting	Weight loss	Duration of wilting	Weight loss
Min.	*Per cent*	*Min.*	*Per cent*
81.5	15.0	80.5	2.8
80.5	9.0	81.0	5.8
29.0	1.7	31.0	1.3
62.0	16.1	61.5	13.3
79.25	21.9	78.0	16.3

time for comparable plants in each experiment. The rate of wilting was uniformly slower in leaves from the diseased plants than in leaves from healthy plants (Fig. 2, A). In this experiment both plants from which leaves had been taken stood side by side in a plot of the experimental field. The healthy plant had 6 healthy pods, no spotted ones; the diseased plant had 4 healthy and 12 spotted pods, the immature ones not being taken into account. Loss of weight in leaves is plotted against duration of wilting in minutes. If loss of water is plotted against area of the leaves, almost the same curve results (Fig. 2, B), thus indicating that no differences in thickness of the leaves are interfering. Therefore it is to be concluded that the differences in the loss of water involved physiological differences in the plants.

If one of two genetically and morphologically homogeneous plants is more resistant against wilting than the other, we must conclude that it is or

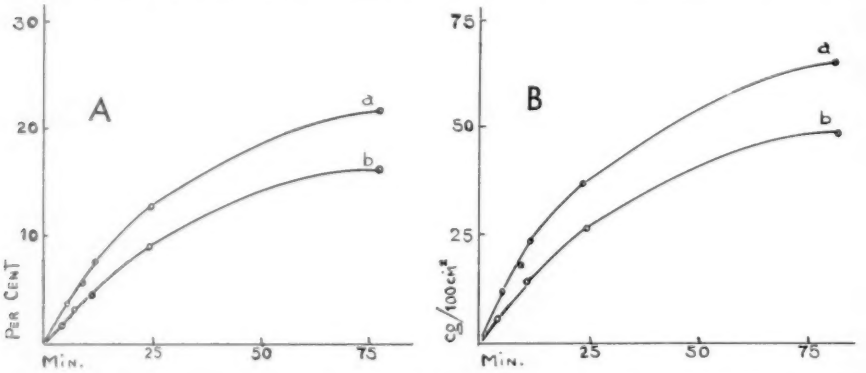

FIG. 2. A. Percentage loss of fresh weight in wilting leaves from (a) healthy pepper plants and (b) pepper plants with pod spot. B. Loss of weight per unit area of leaf.

has been influenced for some time by a factor changing its physiological state in this direction. It seems likely that the factor involved consists of one or several preceding wilting periods endured by the plant. Plants become drought-resistant by repeated wilting. Thus we surmise that pod-spotted pepper plants have been influenced previously by one or several periods of insufficient water. The same cause is assumed generally to be responsible for blossom-end rot of tomatoes. We may repeat that pod-spotting of peppers occurred, in 1940, in the Experimental Field of Ankara, at just the same time as blossom-end rot in an immediately adjacent tomato plot. Both plots had been supplied equally with water: they had been irrigated copiously at intervals of several days and thus had been exposed, in the hot and dry summer climate of Ankara, to great fluctuations of humidity and drought. Another plot of tomatoes, which has received no irrigation at all, produced at the same time a few small but thoroughly healthy fruits. From this, it becomes evident that insufficient water supply in itself does not produce the trouble. It makes the plants hardy, if they survive. Such a condition is not to be taken into account, however, in practical cultivation. On the other

hand, in the small gardens of vegetable growers at Izmir, where irrigation is practiced very diligently and regularly, and therefore periods of drought hardly occur, we almost never found pod-spotting of peppers.

We have tried to produce experimentally pod spots in potted pepper plants by irregular watering. This experiment gave no conclusive results, our equipment being poor for such purposes, and there may have been relatively great fluctuations of water supply in the checks also. In any case, pod-spotting occurred in all pots. It was noted, however, that in the potted plants in which fluctuations of water supply supposedly were greater than in the field, spotting was heavy by the end of June, while in the adjacent field the disease did not appear before the middle of July.

In a small limited area of one field, up to 37 per cent of the pods were spotted. In this case, the subsoil contained refuse of brick and stones from an adjacent building. A disturbed structure of the subsoil may result in fluctuations of the water supply.

A striking feature of the disease is the occurrence of plants with healthy and plants with heavily spotted pods close to each other in one field. For this there is no explanation, for we know practically nothing of the physiological differences of individual plants. Perhaps small differences in the functioning of the roots may play a rôle. One plant was found with all its pods spotted. Its main root had been injured, either mechanically or by an insect.

Although the data we have been able to collect are not wholly conclusive because of limitations of experimental work, we conclude tentatively that in the pod-spotting of peppers both sunscald and blossom-end rot are identical troubles. The damage to peppers is believed to occur during strong radiation of the sun, but only in plants which are or have been influenced by wide fluctuations of water supply.

From the standpoint of practical control of the disease certain facts are obvious. Against sunrays there is no protection. Shading would impair the fruiting and ripening of peppers. But it is possible to avoid fluctuations of water supply. It is necessary to provide moderate but frequent irrigations.

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THE PREVALENCE OF THE WHEAT NEMATODE IN CHINA AND ITS CONTROL[1]

VONG-MAY CHU

(Accepted for publication September 10, 1944)

The nematode disease of wheat, caused by *Anguina tritici* (Steinbuch) Filipjev (= *Anguillulina tritici* (Steinbuch) Gervais and v. Beneden) (= *Tylenchus tritici* (Steinbuch) Bastian) was first found in China in the vicinity of Nanking in 1915. The actual damage caused by this disease was recognized by T. H. Shen and co-workers (15), who reported that the nematode disease of wheat is found in China from north of the Yellow River to south of the Yangtze River. The damage amounts to about 30 per cent at Hsuchow and about 10 per cent at Nansuchow.

The problems concerning distribution and control of the nematode disease have been investigated by the writer since 1933. This paper reports the results obtained up to 1942.

GEOGRAPHIC DISTRIBUTION

The distribution of this disease in China was determined by examining seed samples collected from different districts. It was considered that the results obtained by this method were reliable, since more than 88 per cent of the records obtained from sample examination corresponded with actual counts of diseased heads in several districts. Samples were collected in 1313 counties in 21 provinces throughout the territory of the Republic of China except in Tibet, Mongolia, and Manchuria. A total of 5335 samples of wheat seed were collected and examined. In addition, wheat fields in 165 counties in 20 provinces also were examined. The data show that the wheat nematode occurs in practically every wheat-growing region in China, although its prevalence varies somewhat in different provinces.

ECONOMIC IMPORTANCE

In the provinces of the main wheat belt, more than 13 per cent of the seed samples were contaminated with nematode galls, with percentage of contamination ranging from 0.01 to 5 and mostly from 0.1 to 1.0; and more than 29 per cent of the wheat fields examined were infested, with percentage of infestation ranging from a trace to 50 and mostly from 5 to 10. It was not uncommon for a single mill to discard daily 100 bushels of galled wheat out of 30,000 bushels.

The quantity of nematode galls in the seed, or the number of infected heads in the field is not an accurate index of the damage caused by this disease. Leukel (11) reported that many infected plants are killed in the seedling stage, only a few galls are developed from infected heads, and many

[1] Phytopathology extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

of the galls in the seed are blown out during the threshing process. During the present studies the following facts were observed: (1) When artificially inoculated seed is planted, not more than 54 per cent of the infected seedlings are able to survive until heading. (2) About 80 per cent of the badly infected heads die before maturity and produce few galls or none at all. (3) Not more than 30–90 per cent of the original quantity of galls are found in threshed grain, the remainder having been removed during threshing and refining. (4) The infected heads cannot always be diagnosed correctly. Most of the partially or lightly infected heads, and even some of the severely infected heads can easily be overlooked. In examining 5851 diseased heads of 34 wheat varieties, by dissecting all the flowers in every spikelet, only 22 per cent of the partially diseased heads and 95 per cent of the severely infected ones were diagnosed correctly.

On account of these facts, the actual reduction in yield is far in excess of that indicated by the quantity of nematode galls found in the wheat seed and in diseased heads. Experiments have demonstrated that the actual reduction in yield will amount to 5, 30, 54, and 69 per cent when the weight of the galls in the wheat seed is 0.01–0.09, 2–2.9, 6–6.9, and 8–8.9 per cent of the total weight of the mixture. According to "Crop Reports" published by the Division of Rural Economics, National Agricultural Research Bureau (1), the annual production of wheat in China is about 850,000,000 bushels. The reduction in yield caused by this disease is estimated to be about 11,200,000 bushels.

VARIETAL RESISTANCE

Leukel was the first investigator to test the different varieties of wheat for resistance against the wheat nematode. He (11) failed to find any significant difference in the amount of infection among the different wheat varieties tested, but later (12) found that Kanred was more resistant than a number of other varieties. Leukel's observations were confirmed by S. C. Teng (see 15) who, during 1930–1931, inoculated Kanred seed and also the seed of many Chinese varieties of wheat. None of the Chinese varieties had less than 50 per cent head infection, while only 12 per cent of the Kanred variety was infected.

Experimental studies to determine varietal differences in resistance to nematode were started by the writer in the fall of 1936. At that time, in cooperation with C. Y. Chow of the National Central University, seeds of 72 Chinese wheat varieties were inoculated with galls and planted in the field. From the fall of 1937 up to 1942, the 1677 pure strains obtained by head selection in China, were added to the list, and these experiments were carried on in Chansha, Hunan, and in Kweiyang, Kweichow. Only 5 strains, selected from the tested samples, had little or no infection during the six years' experiments. Those were P.C. 876, P.C. 690, P.C. 633, P.C. 171, and Hsuchow Resistant No. 2. The maximum infection in these varieties did not exceed 0.56 per cent in weight of galls in the threshed grain, though some injury was apparent during the seedling stage. Yields of these strains have

been compared with those of the most common varieties grown in 1940–1942 in Kweichow province. P.C. 171 and Hsuchow Resistant No. 2 are not well adapted to the climatic conditions of the southwestern sections of China where their yields are rather low. The yields of P.C. 633, P.C. 876, and P.C. 690 are as good as, or even better than, the yields of commercial varieties. The main disadvantage is that they mature too late. It is desirable to have early maturing wheat varieties, in order that the wheat fields can be planted to rice on time.

PREVENTIVE MEASURES BY CULTURAL PRACTICES

Crop rotation to reduce the amount of nematode infection has been recommended (1, 7, 11). According to Leukel (11) this is valuable in North America. Fields in the infested areas that previously had grown crops other than wheat for one or two years had less infection than fields that had been planted in wheat continuously. However, the writer doubts that soil infestation is a serious problem in China, where wheat is often followed by rice. Continuous flooding of the rice fields is needed from transplanting to the ripening of the crop. In 4 years' experiments the percentage of nematode infection was highest (3.75 per cent) in the wheat crop grown from clean seed in clean soil, that in the crop grown from clean seed sown in infested soil was less (0.20 per cent), and that in the crop grown from clean seed on soil previously planted to wheat infested with nematode but with an intervening paddy crop was lowest (0.04 per cent). These results indicate that soil infestation is not important, especially when wheat is followed by rice.

CONTROL MEASURES BY SEED TREATMENT

A control program for this disease must be based on the elimination of the nematode galls that are mixed with the seed. Seed treatments that will eliminate the galls and are simple to apply appear to afford the most effective method of control. Various control measures including fanning, screening, hot water dip, chemical treatment, sedimentation by salt brine, flotation in water, and separation by means of an indented cylinder or "trieur" have been suggested by workers in different countries. Unfortunately none of these methods is entirely effective.

Fanning has been recommended by Cobb (see 7) but the effectiveness of this method has not been demonstrated. According to Coleman and Regan (7) 40 to 45 per cent of the galls remain in the wheat even if the fan revolves 850 times per minute. The writer confirmed these results and found that fanning, by the method most commonly used by farmers in China to remove impurities from the grain, resulted in complete removal of the galls from only 6 of 357 samples. The difficulty of separating the galls from the grain by this method is due to the comparatively slight difference in relative weight between the galls and the grain kernels.

Screening is the simplest way for the farmers to achieve gall eradication and is commonly practiced in China as well as in other countries. This treat-

ment is based on the difference in size between the nematode gall and the sound wheat. Sound kernels of *Triticum polonicum* and of the wheat variety Hsuchow 405 were accurately measured as were the nearly globular nematode galls. The ratio of kernel length to gall length is approximately 5 : 1 for both wheats; that of kernel width to gall width is 1 : 0.88 in *T. polonicum* and 1 : 0.78 in Hsuchow 405; and that of kernel thickness to gall thickness is 1 : 0.71 in both wheats. In some cases several small galls may fuse to form a compound gall that is always wider and thicker than the wheat kernel. If contaminated wheat is screened or sieved some of the galls fail to pass through the sieve or a large quantity of small grains pass through. The writer found that screening 390 grams of wheat containing 0.92 per cent of galls, by a method generally used by farmers, reduced the total weight of grain to 342 grams, while 0.05 per cent of the galls remained in the mixture. These results confirm those of Coleman and Regan (7) and Jones and his coworkers (10).

The use of brine to remove the lighter infected grains from wheat seed was independently worked out by Müller in Germany and by Jackzewski in Russia in 1904. Müller used 30 per cent sodium chloride, while Jackzewski used 20 per cent sodium chloride. It was first applied in the control of wheat nematode in North America by Byars (5) in 1919. The considerable difference in specific gravity between the grain and the nematode gall, makes it possible to separate the two bodies. Although this method is considered very effective as a control measure, it is impractical in China on account of the scarcity and expense of salt in many sections of the country.

Coleman and Regan (7) suggested a method to remove the galls from sound wheat by floating infected wheat in water. Since the specific gravity of galls is 0.8125 it is not necessary to increase the density of water by using table salt. If the galls are kept in water for some time, they will imbibe enough water to sink. Observations made with galls collected from different regions show that the galls will float on the surface of fresh water for about five minutes. Consequently, the fresh water method will not be effective unless the operator can remove the galls right after the contaminated wheat is poured into the water. Although it is possible by this method to remove 96 per cent or even 100 per cent of the galls in the wheat, as well as to eliminate most of the seeds of many obnoxious weeds, it cannot be generally practiced in China for a number of reasons. The drying of moist grain is the most difficult problem, requiring considerable space for spreading and constant attention in order to avoid heating and sprouting of the grain. This is especially serious in southwestern China where the regular seeding time of the wheat crop occurs during the autumn rainy season. Another disadvantage of this method is the need for a considerable amount of water. This is a limiting factor in the mountainous districts where it is difficult to obtain water.

Since there is a difference in the heat resisting ability between wheat and the nematode gall, Bessey (2) recommended the hot-water treatment to

remove the source of infection. Marcinowski (13) also proved that the nematode galls in a grain mixture can be destroyed by steeping the mixture for 10–12 minutes in water at 54–56° C. Byars (4, 5) and some other investigators (7, 10, 11) have advocated Johnson's modified hot-water method (9) which involves presoaking the seed in cold water for several hours, after which it is dipped in water at 54° C. for 10–15 minutes. The writer's experiments also demonstrated the necessity of presoaking. Presoaked galls are destroyed when kept in water at 50° C. for 10 minutes or at 55° C. for 5 minutes, whereas the dry galls can withstand the same temperatures and dipping periods. The difficulty in obtaining large quantities of hot water, and the same difficulties encountered with the floating method, make the hot-water treatment impractical for general use in China.

Chemical control measures are based on the difference in resistance against the toxic action of chemicals between wheat and the nematode gall. Davaine (8) recommended soaking wheat in 0.6 per cent sulphuric acid for 24 hours. The results of Pennetier (14), Byars (6), and Marcinowski (13), obtained with different disinfectants, agree with those of Somerville (16) who decided that the contaminated seed could not be thoroughly disinfected without injuring the wheat. The writer also experimented with some common disinfectants such as sulphuric acid, corrosive sublimate, copper sulphate, carbolic acid, and formaldehyde. Of these, the corrosive sublimate appeared most effective and copper sulphate the least. Experimental data indicate that all or nearly all the nematodes are killed after 3 hours in a 1 per cent solution of corrosive sublimate, after 4 hours in a 2 per cent solution of carbolic acid, or after 5 hours in a 5 per cent solution of formaldehyde; but they survive more than 2 hours in a 5 per cent solution of sulphuric acid and 5 hours in a 5 per cent copper sulphate solution. The resistance of galls against the action of certain chemicals varied according to their state of dryness. Dry galls are less resistant to chemicals than galls that have been presoaked. For example, when dipping dry galls in a 2 per cent solution of formaldehyde, 5 per cent survived 5 hours, but when presoaked galls were treated with a similar solution for the same time, 17 per cent survived. It is quite possible that presoaking diminishes the disinfectant qualities of the solutions. Braun (3) believes that presoaking causes the seed cells and cell walls to become saturated with water, so that the disinfectant cannot enter the organism in sufficient concentration, and the amount of disinfectant solution absorbed is also greatly diminished.

Machines for Seed Treatment

Recently Jones and coworkers (10) developed a method to separate mechanically the nearly globular galls from the oval wheat seed. A common weed-eradicating machine, a so-called "trieur," was constructed with a fully and hemispherically indented cylinder. According to these authors, their method was approximately 98 per cent effective. It is less expensive to operate than the brine treatment, it requires less skill than the hot-water

method, and is not so laborious as the water-flotation method. It may be considered, therefore, as the most reasonable and practical means to control the wheat nematode. Unfortunately the so-called "trieur" cannot be obtained in China and although this machine removes 96.7–98.9 per cent of the infectious bodies present in the wheat, it is probable that the 3.3–1.1 per cent of galls remaining may start a new epidemic.

During 1938 the writer in cooperation with S. T. Tsie, senior technician in the Department of Rural Engineering of the Kweichow Provincial Bureau of Agricultural Improvement, designed a wooden machine now known as the "wheat-nematode eliminator," which separates the nematode galls from the sound grain. It is easily constructed and the cost is within the means of the farmer's purchasing power.

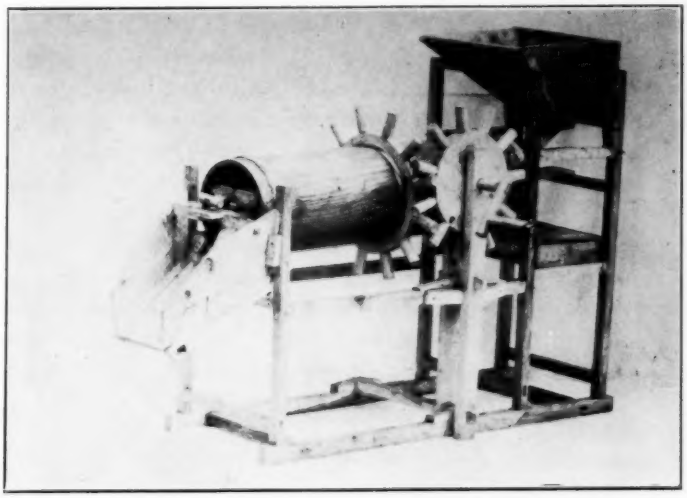

FIG. 1. Photograph of wheat-nematode eliminator.

The construction, process of manufacture, and directions for use of this machine were published in 1940 (17). Since then some improvements have been made. The improved machine is now manufactured in large quantities and is used extensively. It is made entirely of hard, light, durable, and finely grained wood, such as catalpa. It consists of a grainhopper, a grain conduct, a separating cylinder, a gall rejector, a gall receiver, a seed chute, a rotator, and a frame (Fig. 1). The separating cylinder, 80 cm. long and 25 cm. in diameter, is supported on the frame in a slightly inclined position by anterior and posterior bearings. It is turned by a rotator which consists of two ratchets, one centrally attached to the circumference of the cylinder, the other fixed on the frame at a right angle to the former. The inner-wall of the cylinder is densely and evenly indented with hemispherical concavities 4 mm. in diameter. The gall receiver is placed inside the cylinder and consists of a V-shaped tray attached to the frame by two removable bottom plates.

The V-shaped tray is placed in the cylinder so that the top of the tray will be at a height approximately two thirds of the radius above the center. The tray has a length equal to that of the cylinder and has two holes through which the galls are removed by means of a gall ejector, operated by hand, which pushes or pulls the galls to the hole in the anterior end of the tray. The galls drop through the gall rejector, a tubular chute.

The grainhopper is filled with infected wheat, and by means of the adjustor a reasonable quantity of grain falls successively through the gate into the grain conduct, and then drops to the bottom of the cylinder. The rotator turns the cylinder evenly, the grains move gradually to the anterior end of the cylinder, and the galls are separated from the grain and lifted in the cylinder concavities to the top of the tray. The galls fall through holes of the cylinder wall into the tray of the gall receiver. The ejector is then drawn to collect the galls and permit them to fall through the anterior hole in the tray into the rejector chute. The cleaned seeds move slowly to the anterior end of the cylinder and finally fall on the grain chute, which discharges them into a suitable container. A single worker may separate 34 to 45 bushels of grain within 10 hours. For the successful operation of this machine the degree of inclination of the cylinder should not be over 1.5 per cent; otherwise the separation of the galls will not be complete. Experiments have shown that only 96 per cent of the galls are removed when the cylinder is inclined in 2 per cent of its length, while the efficiency of separation may be increased to 99.7 per cent or 99.95 per cent as the inclination is decreased to 1.5 per cent or 0.5 per cent. The most satisfactory results have been obtained by regulating the speed from 60 to 95 revolutions per minute. Though the speed of revolution does not greatly affect the efficiency of gall separation, considerably more grain is wasted if the machine rotates too fast.

Several demonstrations of this equipment were given in different districts in Kweichow province during 1939–1942. In the 1941 harvest in Kweiyang and Wheisui 99.99 per cent of the plants sown from treated grain were healthy, and on the average the treatment for nematode increased the yield by 15 per cent. After this excellent showing, the writer and his coworkers conducted a demonstration campaign in districts of Kweichow province, where the wheat nematode is prevalent. A total of more than 8,000 acres were sown with mechanically cleaned seed, resulting in a yield increase of 25,000 bushels.

CONCLUSIONS

During the last 3 years various comparative experiments have been made in the methods used to control wheat nematode in China.

Fanning is practically useless since treated seed yielded 2.7 per cent galls, while in the check plot 2.4 per cent of galls were found.

Screening gives good results in controlling the nematode but may result in the loss of some of the grain.

Hot water varies considerably in the efficiency of disease control. In

some cases it reduced the percentage of infection to 0.01 per cent but in other cases as high as 4.3 per cent of galls were found.

Water flotation is efficient. This treatment reduced the disease to 0.13 per cent in contrast to 2.3 per cent in the untreated grain, and it also increased the yield by 14 per cent over that of the check plot.

Salt brine sedimentation has completely eliminated the nematode galls in treated seed lots. If there were no need to consider the expense of salt, this would be one of the most effective control measures for wheat nematode, and would compare favorably with the mechanical separation method using the special wooden gall-separating machine.

In the three years' experiments, the wheat-nematode eliminator machine has been the most effective and useful means for controlling the disease in China.

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EFFECT OF LOW TEMPERATURES ON SURVIVAL OF PHYMATOTRICHUM OMNIVORUM[1]

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(Accepted for publication November 28, 1944)

The root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, has long been recognized among plant pathogens as one that is favored by warm weather, although it was known also that the fungus continues spreading slowly during winter along the roots of cotton plants whose tops have been killed by frost (15). Several workers determined the effect of temperature on growth of the fungus in the laboratory. Minimum, optimum, and maximum temperatures for production of sclerotia were reported (5) as 18°, 29°, and 36° C., respectively. Rogers (13) found that growth and production of sclerotia by *P. omnivorum* was greatest about 27° C., and still occurred at 11° C.; there was no growth at 3° C., but the fungus was not killed by exposure to this temperature for 60 days. Virulence of the fungus was retained longer in naturally-infected cotton roots stored at 1° to 2° C. than in roots stored at higher temperatures (16). Previous studies thus agree that *P. omnivorum* grows best at 27° to 29° C. but apparently is not injured by rather long exposure to temperatures just above freezing. There appears to have been no exploration of the effect of more intense cold on the fungus prior to the experiments reported herewith.

Many other fungi which occur in warm climates are found in cold climates also, and survive exposure to very low temperatures. Kärcher (7) listed a group of fungi (including *Schizophyllum commune, Armillaria mellea, Aspergillus niger,* and *Penicillium glaucum*) which in malt agar cultures survived exposure for 8 days at – 70° C., and for 13 hours at – 183° to – 192° C. With the brown-rot fungus, *Sclerotinia* (*Monilinia*) *fructicola,* Bartram (2) found some spores and cultures to survive winter temperatures as low as – 32° C., and Brooks and Cooley (3) reported slow but measurable rot of apples at 0° C.

The question has arisen on several occasions as to whether *Phymatotrichum omnivorum,* although favored by higher temperatures, might not also withstand exposure to severe cold. The practical question is whether this spectacularly destructive fungus would survive if introduced into areas north of the present range, or whether it might succumb to low soil temperatures during the winter.

MATERIALS AND EXPERIMENTAL CONDITIONS

Since *Phymatotrichum omnivorum* overwinters either as vegetative mycelium on roots or as sclerotia in the soil, both stages of the fungus were used in the experiments. The material, uniformly of isolate 24, was as follows:

[1] Published with the approval of the Director as Technical Contribution No. 865 of the Texas Agricultural Experiment Station.

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A. Vegetative growth in young slants, started 20 days before exposure. Cultures were on potato-dextrose agar in small tubes of 12-mm. diameter. After the period of exposure, viability was determined by making transfers from the upper half of the slants, away from the original inoculum.

B. Masses of sclerotia, produced and still in place on the walls of flask cultures (6). The flasks were placed in the various temperature compartments, and removed briefly at appropriate times for transfers to agar slants. Such transfers were made always with small portions taken from areas in the large sclerotial masses (often as large as $1 \times 3 \times 12$ cm.) that had not been disturbed previously, so each transfer was from a new and comparable portion of the sclerotial mass.

C. Cut portions of sclerotial masses, produced in flasks, placed aseptically in agar slants just before exposure to the test temperatures. The sclerotial mass was cut into pieces about $1 \times 1 \times 2$ mm., and these portions placed on the agar slants about 15 minutes before the tubes were installed in the various temperature compartments. After exposure, tubes were taken to the incubator room, where possible growth could be observed without further manipulation.

D. Cut portions of sclerotial masses, in moist soil. Portions cut as for C were placed in small cork-stoppered vials (2×5 cm.) of Houston black-clay-surface soil, adjusted to 34.4 per cent moisture content on an oven-dry basis. After exposure to the test temperatures, the vials were taken to the incubator room, where possible development of strands of the fungus along the walls of the vials could be readily observed, just as in the larger soil-chambers used to determine the viability of the fungus in infected roots (15).

Temperatures used in the experiments were provided as follows:

Freezing compartment,[3] in which cultures were placed inside a double-corrugated-cardboard box, itself inside a metal container. Throughout the inner box, the temperature to which the fungus was exposed was rather uniformly $-13°$ C. (8.6° F.).

Shelf in refrigerator, at 5° C. (41° F.).

Shelves in incubator room, at 28° C. (about 82° F.).

All final observations of viability of the fungus were made at 28° C., following exposure of material to lower temperatures for the periods specified. Cultures were held for several months before discarding the tubes or vials with no growth.

The periods of exposure as tabulated refer to the total time that the containers remained in the temperature compartments. Particularly at the lowest temperature there was undoubtedly a lag, perhaps of several hours before the fungus material itself cooled to the compartment temperature. Thermometers installed inside individual tubes, flasks, or such containers, and observed when material was removed, showed that the experimental temperatures were reached before the fungus was killed, but the lethal time

[3] The writer was indebted to Dr. Jessie Whitacre, Chief of the Division of Rural Home Research, Texas Agricultural Experiment Station, for making space available for this work.

of exposure presumably was somewhat less than the recorded time inside the compartment.

RESULTS

Three types of material, young cultures, sclerotial masses still in place on the walls of flasks in which they were produced, and portions of sclerotial masses resting on agar slants, were exposed for periods of from 18 hours to 50 days to the 3 experimental temperatures (Table 1). Irrespective of the type of material, *Phymatotrichum omnivorum* was killed by exposure to −13° C. for 2 days or longer, while some growth occurred with each type of material that had been exposed to this low temperature for only 18 hours.

TABLE 1.—*Survival of Phymatotrichum omnivorum in the form of mycelial cultures, sclerotial masses, and small portions of sclerotial masses on agar slants, at the temperatures indicated (All run in duplicate, with results similar except for one pair as noted)*

Period of exposure	Growth (+) or lack of growth (−) from following material, after exposure to the temperatures indicated								
	20-day agar slants			Sclerotial masses in original flasks			Sclerotia on agar slants		
	−13° C.	5° C.	28° C.	−13° C.	5° C.	28° C.	−13° C.	5° C.	28° C.
18 hours	+[a]	+	+	+	+	+	+	+	+
2 days	−	+	+	−	+	+	−	+	+
4 "	−	+	+	−	+	+	−	+	+
7 "	−	+	+	−	+	+	−	+	+
12 "	−	+	+	−	+	+	−	+	+
19 "	−	+	+	−	+	+	−	+	+
26 "	−	+	+	−	+	+	−	+	+
50 "	−	+	+	−	+	+	−	+	+

[a] Growth from only 1 of the duplicate cultures.

Refrigeration at 5° C., even for a prolonged period, did not reduce growth of the fungus after transfer to 28° C. However, at 5° C. no visible growth occurred on agar slants that had been seeded with portions of sclerotial material, although the later growth after transfer to 28° C. proved that this material was viable. At 5° C. evidently growth was prohibited but the fungus was not killed.

Since in the first experiment the freezing temperature had killed the fungus in all but the shortest period of exposure, a second series was run with a single type of material, removed from the temperature compartments at more frequent intervals. Cut portions of sclerotial masses on agar slants (material C) were exposed to low temperatures for periods from 15 hours to 7 days, with the results given in table 2. Again, the refrigeration at 5° C. had no deleterious effect. Exposure to −13° C., on the contrary, reduced viability within 15 hours so that not more than 2 of 3 slants removed at any one time showed growth later, and by 39 hours had killed the remaining material.

In a third experiment, the effect of freezing under more natural conditions was considered by use of sclerotial material placed in vials of moist

TABLE 2.—*Survival of sclerotia of P. omnivorum, placed on agar slants and exposed to the temperatures indicated (Run in triplicate)*

Period of exposure	Growth (+) or lack of growth (−) after exposure to	
	−13° C.	5° C.
15 hours	+[a]	+
17 "	+[a]	+
19 "	+[a]	+
21 "	−	+
24 "	+[a]	+
39 "	−	+
2 days	−	+
3 "	−	+
4 "	−	+
7 "	−	+

[a] Growth from only 2 of the triplicate cultures.

Houston soil. This soil had not been sterilized and was fairly comparable to a favorable soil under field conditions. At −13° C. the sclerotia in the moist soil died within 19 hours (Table 3); the sclerotial masses left in the flasks survived for this period but were killed within 21 hours. At 5° C. there was uniformly no loss of viability during the 5-day-maximum exposure.

DISCUSSION

The three experiments reported have shown that *Phymatotrichum omnivorum* is highly sensitive to a freezing temperature. In none of the forms tested was the fungus viable after exposure to −13° C. for longer than 24 hours.

This sensitivity to a low temperature presumably explains the northward limitation of the range of Phymatotrichum root rot. Figure 1 shows the general area in which root rot has so far been found, from a point in southern Utah southward into Mexico (where the southern limit is yet to be estab-

TABLE 3.—*Survival of P. omnivorum in the form of sclerotial masses, dry on the walls of flasks where produced, and as small portions of these masses in vials of moist soil, at the temperatures indicated (Run in duplicate)*

Period of exposure	Growth (+) or lack of growth (−) of the following material, after exposure to the temperatures indicated			
	Sclerotial masses in original flasks		Sclerotia in moist soil in small vials	
	−13° C.	5° C.	−13° C.	5° C.
0 (check)	+	+	+	+
19 hours	+	+	−	+
21 "	−	+	−	+
24 "	−	+	−	+
26 "	−	+	−	+
28 "	−	+	−	+
43 "	−	+	−	+
52 "	−	+	−	+
3 days	−	+	−	+
5 "	−	+	−	+

lished), and from the eastern margin of Texas westward to southern California (4, 8, 9, 10, 11, 12, 14). Superimposed on this map are two isotherms, a solid line representing an annual mean temperature of 60° F. (from Climate and Man, 1, p. 703), and a broken line delimiting the area with a lowest observed temperature of – 10° F., between 1899 and 1938 (1, p. 709). These isotherms, particularly the latter, follow rather accurately the northern limit of occurrence of root rot from Arkansas west to Nevada. At the extreme western and eastern limits of this area, other conditions appear to limit spread and the temperature no longer seems the important factor.

It may be noted that the correlation of occurrence of root rot with the accumulated weather records is good also with different summations of temperature data. An average January temperature of 40° F. (1, p. 704), or an average July temperature of 80° F. (1, p. 705), or an average annual minimum temperature of 5° F. (1, p. 707), agree nearly as well with the limit

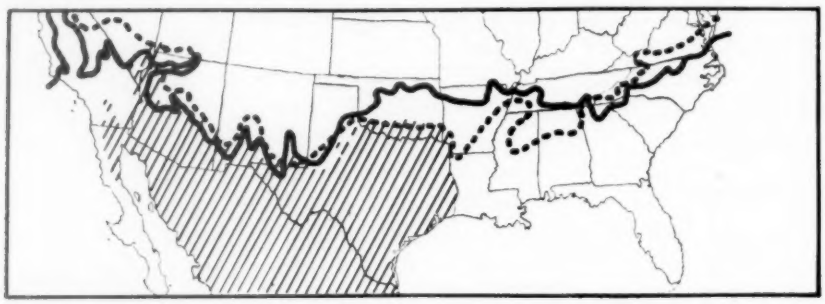

FIG. 1. Correspondence of root-rot area with recorded (1) temperature data. Shaded area, locations in which Phymatotrichum root rot has been found; solid line, annual mean temperature of 60° F.; broken line, lowest observed temperature between 1899 and 1938, – 10° F.

of root-rot areas as the 60° mean temperature. An average annual snowfall of 10 inches (1, p. 727) and an average frost-free period of at least 200 days (1, p. 746) are only somewhat less in agreement.

Soil-temperature conditions suitable for continued survival of *Phymatotrichum omnivorum* thus have occurred over a period of years within the climatic area delimited by the temperature indices mentioned. In other words, root rot has persisted where the temperature has not fallen below – 10° F. (– 23° C.), where the annual mean temperature has been 60° F. (15.6° C.) or higher, and where the frost-free period has averaged at least 200 days a year. As was noted earlier (4), the disease is increasingly prevalent, within similarly favorable soil areas and with comparable rainfall, southward from the northern limit chiefly discussed here, along with further increase in temperature. It is quite possible that the final northern limit is set by the occasional very cold periods during which the fungus may be exposed briefly to unusually low (and lethal) temperatures in the soil, in line both with the sensitivity of *P. omnivorum* to short periods of freezing in the laboratory, and the rather precise delimitation of the northern limit of the root-rot zone by the – 10° F. line (Fig. 1) as already noted.

It seems unlikely that *P. omnivorum* will survive in more severe climates unless some considerable change in sensitivity of the fungus to cold should occur. The data presented suggest little reason for alarm as to the possibility of much advance of the fungus northward, either by slow growth through the soil (such subterranean spread may be at a maximum rate of about one mile in 100 years) or by more rapid introduction on the roots of diseased plants.

SUMMARY

Phymatotrichum omnivorum whether as vegetative growth on agar slants, in large sclerotial masses high on the walls of flask cultures, or as portions of sclerotial masses on agar slants or buried in moist soil, did not survive exposure in the laboratory to a temperature of −13° C. for more than 24 hours. At 5° C., growth was prevented, but there was no reduction in viability even after 50 days.

The northern limit of natural occurrence of root rot corresponds generally with several summaries of recorded temperatures, and particularly well with the line at which lowest observed air temperatures reached −10° F. (−23° C.). Coupled with the sensitivity to cold shown by the fungus in the laboratory experiments, this suggests that northward distribution of Phymatotrichum root rot has been limited by prevailing temperatures, and that the fungus is not likely to become established north of the present root-rot area.

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INFLUENCE OF TEMPERATURE ON THE DUTCH ELM DISEASE IN POTTED AMERICAN ELM

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(Accepted for publication December 4, 1944)

The Dutch elm disease pathogen, *Ceratostomella ulmi* (Schwarz) Buisman, grows little or not at all on artificial culture media at temperatures of 33° C. and higher, and at 10° C. and lower it grows poorly.[2, 3, 4] Because of this behavior the influence of temperature on disease development in trees artificially inoculated with the pathogen was studied. The methods used and the information obtained are briefly summarized.

In the summers of 1940, 1941, and 1942, about 200 potted American elms (*Ulmus americana* L.) were used in 6 different temperature experiments. To provide for uniform response to temperature and to the disease the trees were grown from cuttings; in height they ranged from 2½ to 7 feet and in age from 3 to 4 years. Except for those used in one experiment all trees had completed ⅔ to ¾ of their terminal growth; the excepted trees had recently completed terminal growth before being used. In general, the vigor of all trees was good.

Six different temperature chambers were used, and, in addition, outdoor conditions were utilized. The chambers were 7 to 8 feet high by 8 to 10 feet square. The temperature series ranged from about 10° C. to about 35° C. The minimum and maximum temperatures and relative humidities recorded for each chamber in the series, and for outdoors, from that of lowest temperature to the highest were respectively: A. 9.5° to 12.0° C., 75 to 85 per cent; B. 15.0° to 16.0° C., 80 to 94 per cent; C. 18.5° to 21.0° C., 76 to 85 per cent; D. 12.5° to 20.0° C., 62 to 100 per cent (outdoors); E. 21.5° to 25.0° C., 61 to 78 per cent (basement corridor); F. 26.0° to 29.0° C., 69 to 84 per cent; G. 32.0° to 37.0° C., 66 to 79 per cent. Except for the basement corridor, blowers circulated the air in the chambers and each was lighted by a 150-watt Mazda lamp.

Inoculum consisted of distilled water suspensions of conidia[5] from 10- to 12-day-old cultures of a virulent line of *Ceratostomella ulmi* grown in Petri dishes on sterile, cellulose, beer-glass coasters soaked with potato-dextrose solution. The density of the spore suspension was adjusted to about ½ million spores per ml.

Inoculation was accomplished by means of a 10-ml. hypodermic syringe equipped with a 11/16-inch, No. LN special Becton-Dickinson & Co. needle

[1] The use of facilities made available by the Boyce Thompson Institute for Plant Research, Yonkers, New York, is gratefully acknowledged.

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[5] Spores taken from coremia of the Graphium stage of the fungus.

having the delivery aperture on its side near the point. After loading the syringe with spore suspension the needle was inserted underneath the bark, on a tangent with the trunk circumference, at one point on each of two sides but at slightly different levels and about 14 inches above the soil level. As the needle was forced underneath the bark care was taken to injure some of the peripheral vessels of the wood to assure ingress. Two ml. of spore suspension were delivered at each point. The method of inoculation used was the most expeditious and effective of many earlier tried and it had no appreciably injurious effects upon the trees.

Usually the different groups of trees were placed in the temperature chambers 12 to 24 hours before inoculation. In one experiment the trees were inoculated just previous to their transfer from outdoors into the chambers. The experiments were performed during June and the first half of July in the different years.

Exposure of the trees varied from one week to one month in the different experiments; in some experiments the period was fixed and in others it was determined by the development of the disease. Data concerning wilt symptoms, temperature, and humidity were recorded each day, and at suitable intervals representative trees from the different treatments were cut and examined for discoloration in the wood. After the exposures all remaining trees in a given experiment were held outdoors until leaf fall, or until resumption of growth the following spring, after which they were cut and examined.

Air temperature exerted a marked influence on the development of the disease in the artificially inoculated, potted elms. The range of temperature generally favoring disease development extended from about 15° to 29° C. Trees exposed to temperatures that fluctuated daily between 26° and 29° C. had 100 per cent wilt in 3 to 5 days, and the extent of wood discoloration showed that invasion by the pathogen was complete or nearly so. Exposure of inoculated trees to temperatures of 32° to 37° C., or at 9.5° to 12° C. definitely retarded or even inhibited wilt; usually at least 2 weeks elapsed before wilt appeared and then it occurred only in minor amounts. The invasive activities of the pathogen, as marked by discoloration in wood, likewise were almost entirely suppressed by the high and low temperatures.

The results obtained by shifting inoculated trees from temperatures favorable to the pathogen to those unfavorable and vice versa also emphasized the influence of temperature on the development of the disease. For example, symptoms were delayed 2 to 5 days in inoculated trees that were held at about 27° C. for 4 days and then shifted to a chamber held at about 10° C. On the other hand, in two groups of inoculated trees, each held for 4 days at a temperature distinctly unfavorable for the pathogen, the symptoms were speeded by 2 to 4 days over those of inoculated checks (check trees held constantly at unfavorable temperatures) by shifting them to the more favorable temperature of 27° C. The removal of inoculated trees from temperatures unfavorable for the development of the disease, such as

10° C. and 32° to 37° C., to the outdoors after 1, 2, and 3 weeks exposure ultimately resulted in complete wilting and extensive fungous invasion if the trees were in an active state of terminal growth at the start. If the trees had completed their terminal growth before exposure to unfavorable temperatures, the disease often was definitely suppressed in some trees and never developed at all in others even after they were removed to the outdoors.

In trees that had completed terminal growth shortly before they were inoculated, the disease was completely inhibited by a 15-hour exposure at night (5 P.M. to 8 A.M.) at 32° to 37° C. alternated with a daily 9-hour, outdoor, daytime (8 A.M. to 5 P.M.) period at temperatures that ranged from 10° to 33° C. The reverse treatment, *i.e.*, indoors at high temperature during the day and outdoors at cooler temperatures at night, permitted complete wilting of similar trees in from 14 to 26 days. Inoculated trees kept constantly outdoors as checks during the same period wilted completely within 2 weeks; inoculated trees kept constantly at 32° to 37° C. as checks did not become diseased but their foliage was severely injured by the high temperature. Foliage of trees in the groups that were alternated daily between the two temperatures was not injured by high temperature.

The results, which are not in accord with those of Buisman,[6] show that pathogenic activities of *Ceratostomella ulmi* in potted elms may be definitely altered by temperature. Temperatures known to suppress saprogenic activity, likewise were inhibitive to pathogenic activity, while a temperature that was optimum for the organism as a saprogen also speeded its activity as a pathogen.

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[6] Buisman, Christine. Verslag van de phytopathologische onderzoekingen over de iepenziekte, verricht in het Laboratorium, Willie Commelin Scholten, gedurende 1931. Tijdschr. Plantenz. 38: 17–36. 1932.

SOME ETIOLOGICAL ASPECTS OF MEALYBUG WILT[1]

WALTER CARTER

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The writer's first paper on the etiology of mealybug wilt (1) concluded that the evidence "pointed to a nonliving toxic insect secretion as causal." In later papers (2, 3, 4), this conclusion was reaffirmed, while in 1939 a summary of the investigations was included in a review of injuries to plants caused by insect toxins (5). Many of the findings reported herein were made in connection with a study of the effect of fertilizer on susceptibility of pineapple plants to mealybug wilt.

Topics for discussion in this report include: symptom expression and the period for the development of symptoms; the recovery of plants from mealybug wilt and the relation of repeated mealybug infestation thereto; the susceptibility of recovered plants to a second infestation of mealybugs; the localization of wilt in experimental plots; the effect of sunlight on susceptibility; and finally, some data on infested planting material and an infestation method.

SYMPTOM EXPRESSION

Designation of developing symptoms into four classes with a fifth indicating a recovery stage has proved practicable. Stage 1 is the preliminary reddening of the leaves. In stage 2, a definite color change from red to pink occurs, as well as the reflexing of the leaf margins. In stage 3, the affected leaves have lost their turgor and have drooped, while in stage 4, the affected leaves have dried up for the greater part of their length. A relatively small proportion of cases develop to stage 4, stage 3 usually being followed by the recovery stage. After plants have recovered and the oldest affected leaves become senescent, the youngest affected leaves, having then grown out, will be wilted at the tips only, while the new center leaves are apparently growing normally. This stage has been designated by the initials TWNC. If plants wilt when showing inflorescence or green fruit, the drying up of these is accompanied by the development of the first three wilt stages in the innermost leaves or the bracts at the base of the developing fruit.

Following the course of the disease over many thousands of plants, the expression of symptoms on one or two leaves only of the plants has been noted from time to time. This limited expression may reach any one of the first three stages of wilt. Some of these cases progress to typical third stage wilt, but in others no further development of symptoms occurs. The incidence of these cases is shown in table 1, and it should be noted that they did not occur in plants having only a short period for the development of symptoms.

[1] Published with the approval of the Acting Director as Technical Paper No. 154 of the Pineapple Research Institute of Hawaii, University of Hawaii.

TABLE 1.—*The incidence of wilt cases in which symptoms were limited to (A) 1 or 2 median leaves only and (B) 1 or 2 median leaves followed by complete wilt*

Age of plants when infested	No. days after infestation	No. of plants		
		With wilt	With limited wilt	
			A	B
Test 1	55	1,065	0	0
5 months	68	3,559	0	0
	104	820	5	31
	134	160	10	5
	165	23	8	0
Test 2	43	34	0	0
5 months	49	353	0	0
	58	1,070	0	0
	70	3,637	0	0
	78	1,064	0	0
	105	286	23	75
	125	92	4	0
Test 3	56	3	0	0
9½ months	63	102	0	3
	82	298	0	40
	90	104	0	32
	125	336	1	46
	145	162	1	13
	181	154	2	19
	215	74	2	0
	246	32	2	0

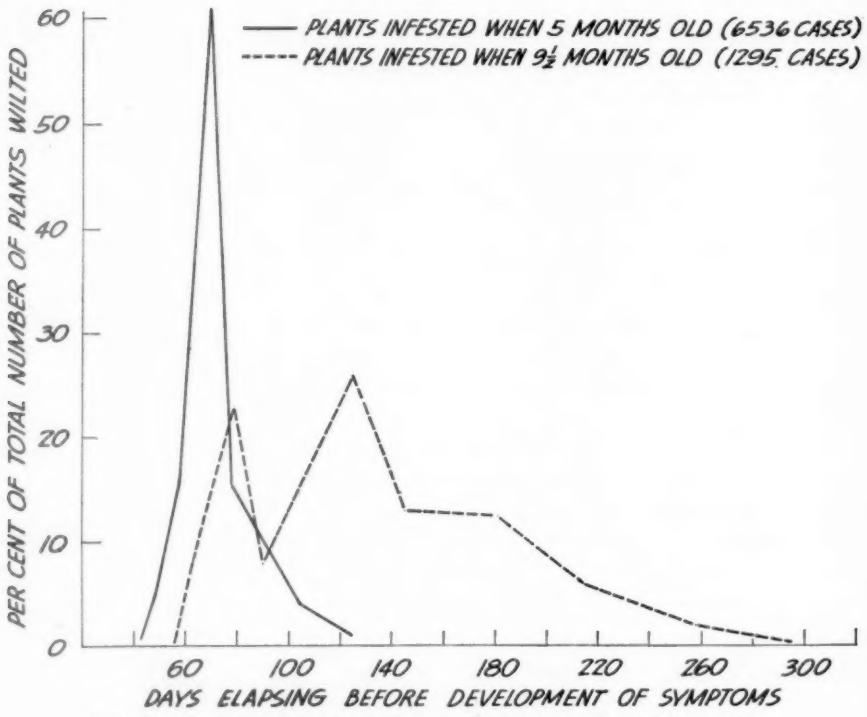

FIG. 1. Period for the development of symptoms of mealybug wilt.

PERIOD FOR DEVELOPMENT OF SYMPTOMS

The term "incubation period" cannot be properly used to describe the period between the feeding of mealybugs and the first symptoms of wilt, since there is no evidence whatsoever of a period of increase for any causal entity. Insofar as the term "latent period" carries the same connotation, the same objection holds. It has been previously indicated that a period of approximately two months is necessary between the insects' feeding and the appearance of typical wilt symptoms, but our information on this has now been considerably extended.

Tables 2 and 3 and figure 1 present the data for two extensive factorial experiments in which detailed records were kept of the development period.

TABLE 2.—*Variation in the time required for the development of symptoms of mealybug wilt and distribution of the 6,536 cases according to factorial treatment. Plants infested when five months old*

Treatment	No. of plants which wilted from 35 to 125 days after infestation							
	35	43	49	58	70	78	105	125
Low Fe	0	9	181	558	1,799	572	134	36
High Fe	0	25	172	512	1,838	492	152	56
Low N	0	11	126	443	1,623	708	203	61
High N	0	23	227	627	2,014	356	83	31
Low K	0	16	161	519	1,858	539	142	36
High K	0	18	192	551	1,779	525	144	56
No chloropicrin	0	9	100	260	1,777	868	189	63
Chloropicrin	0	25	253	810	1,860	196	97	29
Totals for all plots combined	0	34	353	1070	3,637	1,064	286	92
Percentage plants wilted for all plots combined	0	0.52	5	16	56	16	4	1

Table 2 is from a series which was infested when the plants were five months old (7, Factorial Test 2), and the distribution of most of the cases between 58 and 77 days is clearly shown, with a mode at 70 days. It should be noted also that there was a decided tendency for the disease to appear in a shorter time in plants grown at high N levels and in chloropicrined soil. Table 3 presents a similar set of data for a test with plants which were infested at nine and a half months old (7, Factorial Test 3) and shows no clearly defined peak as with younger plants, but rather a scattering over 82 to 181 days, with considerable numbers of cases showing a still longer period. All of the late cases were those in which wilt symptoms first appeared when the fruits were green.

RECOVERY

Frequent reference has been made to this phenomenon. If a wilted plant is kept free of mealybugs, recovery from the center always occurs, although it may be, and usually is, slow if the plant originally went to the fourth stage of wilt. Typically also, the originally wilted leaves remain on

TABLE 3.—*Variation in the time required for the development of symptoms of mealybug wilt and distribution of the 1,295 cases according to factorial treatment. Plants infested when 9½ months old*

Treatment	No. of plants which wilted from 56 to 295 days after infestation										
	56	63	82	90	125	145	181	215	246	258	295
Low Fe	2	53	157	61	177	77	93	28	13	17	0
High Fe	1	49	141	43	159	85	61	46	19	10	3
Low N	2	56	155	60	176	86	97	49	11	13	2
High N	1	46	143	44	160	76	57	25	21	14	1
Low K	0	49	154	58	166	83	90	33	18	14	3
High K	3	53	144	46	170	79	64	41	14	13	0
No chloropicrin	0	39	143	51	165	79	84	36	15	14	1
Chloropicrin	3	63	155	53	171	83	70	38	17	13	2
Totals for all plots combined	3	102	298	104	336	162	154	74	32	27	3
Percentage plants wilted for all plots combined	0.23	8	23	8	26	13	12	6	3	2	0.23

the plant until senescent without loss of symptoms. In recent large-scale experiments under frequent observation, a considerable percentage of plants showed the first stage of wilt only, namely, the slight all-over reddening of the leaves, and on subsequent observation this symptom had disappeared and the plant was apparently normal. Data concerning this observation (Table 4) are from the factorial test 2 of the preceding paper (7), which can be referred to for details of the treatment. There was no apparent relationship between the number of plants wilted in any replication and the number of plants that recovered from first stage wilt. It is clear that there is no relationship between fertilizer treatment and this phenomenon, but it is equally clear that the differences between plots are very significant.

Wilt symptoms are associated with the death of roots, and recovery with

TABLE 4.—*Summary of cases in which symptoms did not progress beyond first stage and recovery was rapid*

Treatment				Total plants wilted	Total plants recovered from 1st stage wilt	Percentage
N1	K2	S1	F1	426	33	7.7
2	2	2	1	374	35	9.4
2	1	1	1	433	71	16.4
1	1	2	1	447	20	4.5
1	1	1	1	361	46	12.7
2	1	2	1	429	8	1.9
2	2	1	1	420	84	20.0
1	2	2	1	399	58	14.5
2	1	2	2	445	14	3.1
1	1	1	2	374	23	6.1
2	2	1	2	444	77	17.3
1	2	2	2	445	33	7.4
2	2	2	2	384	41	10.7
1	1	2	2	349	45	12.9
1	2	1	2	374	30	8.0
2	1	1	2	433	63	14.5

the production of new roots, but the disappearance of the first stage symptoms, which, incidentally, require considerable experience for their correct determination, can perhaps be explained on the basis that in such cases, only a portion of the root system had been affected, or that new roots were being produced distal of the affected zone of the plant during the period for development of symptoms. It must also be evident that if transient first stage symptoms only can appear, it is very probable that damage to the plants might occur without visible symptoms.

Recovery may be affected by nitrogen fertilization (Table 5). These data were obtained in connection with a test of the effect of nitrogen fertilization on susceptibility (7, Nitrogen Test 1) and show that plants in section 1, infested when approximately three months old, recovered and fruited in direct proportion to the amount of nitrogen supplied. Recovery in plants

TABLE 5.—*Effect of nitrogen fertilization on recovery of wilted plants to fruiting stage*

	Nitrogen	Section 1, infested Jan., 1937	Section 2, infested April, 1937	Section 3, infested June, 1937
Percentage of plants wilted	0	72.65	29.57	19.30
	100	73.73	8.77	18.42
	500	68.33	20.69	19.47
	1,000	72.73	19.13	15.65
Percentage of wilted plants fruited	0	24.71	55.88	63.64
	100	33.33	50.00	47.62
	500	47.57	33.33	54.55
	1,000	48.27	27.27	33.33

in sections 2 and 3, infested later at approximately six and eight months old respectively, shows an almost converse trend. Notes on recovery were taken up to November of the plant crop year, or four to five months after the normal harvest season.

The plants which wilted when only three or four months old had only the first flush of roots affected and new roots permitted recovery in proportion to the nitrogen supply. Plants infested and wilted later had had a much larger percentage of the available root "buds" developed and therefore damaged. In the large soft-leaved plants of the higher nitrogen series collapse was complete and "flabby," more so than in the lower N plants which were harder and higher in carbohydrate reserves. The former were much slower in recovering.

A relationship between intensity of infestation and extent of recovery has been noted recently. Two contiguous beds of pineapple plants were infested with mealybugs, one bed three times at monthly intervals and the other bed once only. In both cases 100 per cent of the plants had typical wilt symptoms. The relative recovery of these plants, however, was of quite a different order, the series infested only once showing very real recovery compared with the more heavily and frequently infested series (Fig. 2).

The most logical explanation of this difference is that the more frequently infested plants received three separate successive injuries to the roots which would materially reduce the number of root "buds" available to the plant for recovery. This would seem to be a more logical explanation than to ascribe it to mass action *per se*, since mass action presumably must occur at the same place and time.

Another observation pertinent to this question, however, indicates clearly that mass action is a factor. It used to be the practice when harvesting pineapples to pile the crowns that were cut from the fruits at harvest time

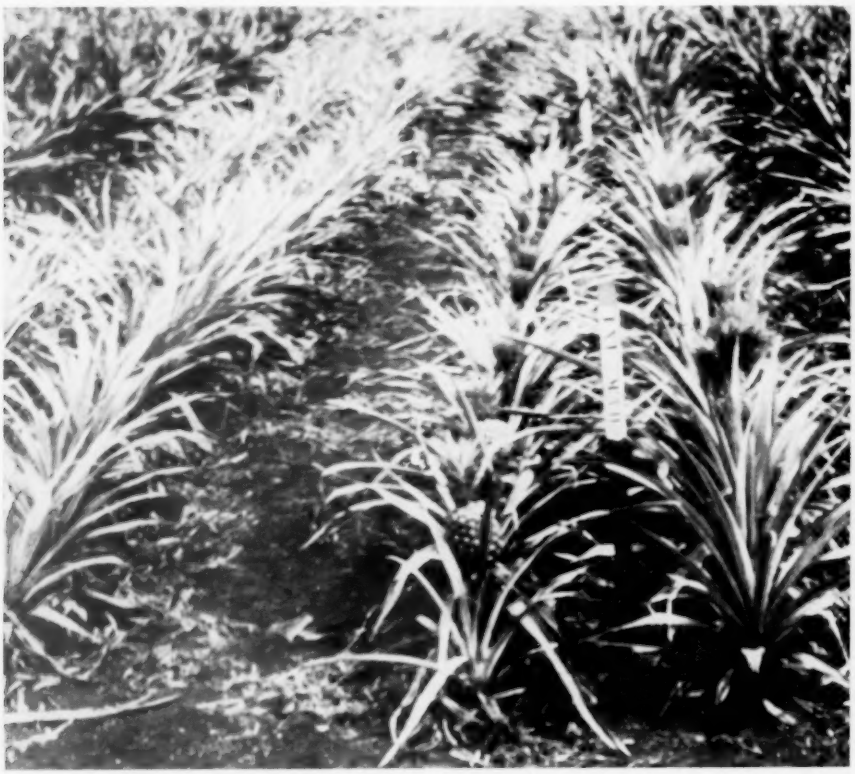

FIG. 2. Recovery from severe wilt in plants infested only once (right) compared with that in plants infested three times at monthly intervals (left).

on top of the developing ratoon suckers at the ends of the beds. This very frequently resulted in wilt in these suckers which became infested from the mealybugs leaving the drying crowns. As it was frequently observed, the wilt at the ends of the beds was out of all proportion to the scattered wilt incidence that may have occurred in the fields from which these crowns were collected. A recent case in point recalled this old practice and gave an opportunity for detailed observation. Figure 3 shows the end of a pineapple bed in which the ratoon suckers are typically wilted. This bed had had the crowns from the fruit harvested in that area piled on it at the end.

Careful examination of the plants from which these crowns came showed not a single case of wilt in any of the plants. It was clear from this that populations of mealybugs inadequate to cause wilt in the plants from which they came were sufficient to do so when concentrated on a few plants at the end of the beds. Experience with field control, however, adds a large body of indirect evidence to these specific observations. Control measures have been developed which, when properly applied, prevent the development of popu-

FIG. 3. First ratoon suckers wilted as a result of piling infested crowns from plant crop fruits at the end of the line.

lations sufficient to cause wilt but they do not eliminate all the bugs. When these controls are discontinued or inadequately applied, these small populations rapidly increase with the result that wilt develops on local areas comparable to that obtaining prior to the development of control measures.

THE SUSCEPTIBILITY OF RECOVERED PLANTS TO A SECOND GROSS INFESTATION OF MEALYBUGS

Detailed data concerning this have been presented in the preceding paper (7), because it was in connection with the second wilting, that fertilizer treatment appeared to have some significant effect. The fact that a recovered plant is just as susceptible to wilt by a second heavy infestation of mealybugs as is one which showed no symptoms following the first infestation, is a clear datum supporting the hypothesis that mealybug toxin does

not reproduce in the plants and that new tissue is toxin-free. The symptoms in the second wilting were precisely the same as in the first.

THE EFFECT OF EXPOSURE TO SUNLIGHT

It has been recognized for some time that light was an important factor in the expression of wilt symptoms. Even in a relatively open greenhouse covered with a heavy grade of reinforced glass, which no doubt radically affects light quality and intensity, conditions are so unsatisfactory for the expression of typical wilt that the use of such houses for laboratory experiments on wilt has long since been abandoned. In four factorially arranged experiments, in two of which the plants were infested when about five months old and in two when the plants were nine and one-half months old, it was evident even to the casual observer that in the plants infested when they were quite young and small, the incidence of wilt on the south side of the two-row bed was greater than on the north side. This observation is supported by the statistical analysis of the data which reveals a significant increase in the wilt incidence on the south side of the bed. Wilt incidence in plants infested at nine and one-half months old, when they had attained considerable size, did not show this significance. These data are in table 6.

TABLE 6.—*Chi-squared* (χ^2) *test of wilt incidence on north and south sides of two-row beds*

Test No.	No. of wilted plants			χ^2	Probability
	North row	South row	Total		
1[a]	2,691	2,934	5,625	10.5	< 0.01[c]
2	3,175	3,374	6,549	6.0	< 0.02[c]
3[b]	659	636	1,295	0.4	< 0.50
4	364	383	747	0.5	< 0.50

[a] Plants infested when five months old.
[b] Plants infested when nine and a half months old.
[c] Significant.

LOCALIZATION OF WILT IN EXPERIMENTAL PLOTS

This localization has been encountered in greater or less degree in every field experiment on mealybug wilt for the last 12 years. Figure 5 is a diagram of all the plants in an experiment on fertilization in relation to susceptibility which was arranged in factorial design (7, Factorial Test 4). All the plants in these plots were infested on the same day with very heavily infested pieces of fruit rind, so that if the plants were equally susceptible at the time they were infested, the distribution of wilt should have been much more uniform. The fact that wilted plants are grouped in localized areas to the extent that this figure shows, suggests again the presence of a factor affecting susceptibility that can only be ascribed to the biological soil complex. Evidence was obtained that these local areas where wilt incidence is high are not stable, when one large field factorial experiment was duplicated exactly both with respect to treatment and location of the plots in the

area. Although localization occurred in both experiments, that in the second one did not coincide with that which occurred in the first. Differences in the physical state of the soil or of the soil itself would seem, therefore, to be ruled out. There is a very clear relationship between root collapse and wilt, and any hypothesis to account for this localization should consider the possibility that the plants in those locations were rendered more susceptible to mealybug wilt as a result of microbial activity.

There is sufficient evidence, however, to indicate that well-developed root systems of themselves are not adequate to reduce susceptibility in young plants, for it has been amply shown that chloropicrin treatment of the soil does not affect susceptibility of young plants to wilt (7) and it is evident that whatever the soil factor may be which affects susceptibility, it is not one which is affected by chloropicrin treatment of the soil. Although no formal tests have yet been made with D–D mixture in this connection (6), there have been sufficient cases of incidental wilt occurring in plots treated with D–D mixture to indicate that this material may have no more effect in reducing susceptibility in young plants than does chloropicrin.

Except for a few specific organisms, very little is known concerning the biological complex in pineapple soils, and still less about species interaction. The best evidence that the state of the soil affects susceptibility is to be found in the observation, frequently repeated, that pineapples grown in virgin land are definitely less susceptible to mealybug wilt than are plants grown in old pineapple areas. This is true whether the area in question is far removed from previous pineapple cultivation or whether it occurs as a result of relocating field margins to include small sections of contiguous virgin land. Less frequently observed has been a similar result in land treated with chloropicrin before planting. Mealybug infestations in the few such cases were not recorded in the early stages of growth of the field, since the observations were not made until the fields were in first ratoon stage and showing dense growth, but the presumption is that they were low.

MEALYBUG INFESTED PLANTING MATERIAL IN RELATION TO LATER SUSCEPTIBILITY OF THE GROWING PLANT

When mealybug-infested planting material is planted in a properly prepared field, most of the bugs usually disappear in a short time.

Experiments were set up to test the influence of such planting-material infestations on the susceptibility of these same plants to wilt following mealybug infestation at a later stage of their growth. The pieces of planting material from 34 mother plants were planted in clones at which time a record was made of the mealybug population on each piece. Twenty-one mother plants had either all or only part of the planting pieces infested with mealybugs at the time of planting, while 13 mother plants had no infested planting material. Shortly after growth had started, the mealybugs on the infested plants were eliminated in the usual manner and all plants were maintained free of mealybugs until experimental infestations were made.

It is clear that the previous infestation of the planting material did not affect the percentage of plants that wilted following later mealybug infestations, for of the 13 mealybug-free clones, 62 per cent of the plants wilted, while of the mealybug-infested clones, 61 per cent of the infested plants wilted and 57 per cent of the mealybug-free plants wilted.

THE FRUIT RIND INFESTATION METHOD

This technique for large-scale infestations consists essentially of selecting infested green fruits, cutting off the rind and dropping small pieces into the hearts of plants to be infested. The method was checked to determine whether mealybug colonies from individual fruits differed in toxicity. The pieces of rind from single fruits were applied consecutively to plants in the row and when wilt had developed, the diagram of the wilt incidence was

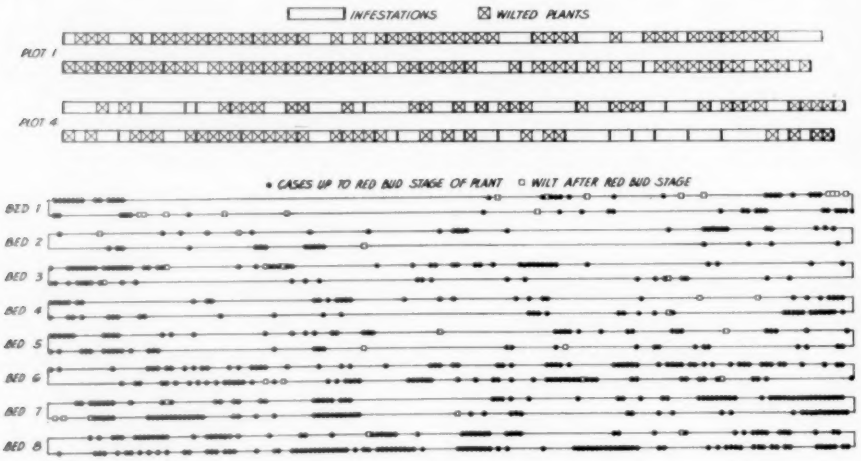

FIG. 4 (top). Diagram showing plants infested with mealybugs by the fruit rind method, using pieces from single fruits to infest consecutive plants in the row.

FIG. 5 (bottom). Diagram showing position of all the wilted plants in a field plot in which the plants had been infested with mealybugs by the fruit rind method with pieces selected at random.

superimposed on that for the single fruit infestation. There was no indication that any differences existed in the toxicity of individual fruit colonies. Figure 4 is a diagram of a typical section of the result obtained for several thousand plants. This figure should be compared with figure 5 which diagrams the total wilt incidence in a large-scale experiment in which pieces of fruit rind from hundreds of fruits were used at random. The patterns of wilt incidence are essentially the same in both figures.

SUMMARY

Observations and data on the etiology of mealybug wilt are presented. Symptom expression can conveniently be designated in 4 progressive stages and 1 recovery stage. Limited expression of symptoms may occur and 1 or 2 leaves only be affected if the period for the development of symptoms is

prolonged. The period for the development of symptoms, which varied from 43 to 295 days, is affected by the age of the plant at time of infestation.

Recovery from advanced stages of wilt occurs but that from first stage wilt can occur with complete loss of symptoms. Recovery may also be affected by N fertilization but the age of the plant at time of infestation is a factor. Frequent mealybug infestation adversely affects recovery. There is additional evidence that mass action is a factor and that sub-wilting mealybug colonies, when joined, will cause wilt.

Recovered pineapple plants are susceptible to later mealybug infestation and will wilt a second time, with typical symptoms. Reduced wilt incidence in glass houses and on the north side of two-row beds suggest that light exposure is a factor in susceptibility. Wilt in field plots is localized and diagrams showing the pattern of wilt incidence are presented. When infested planting material was planted and the mealybugs removed, no effect of those mealybug colonies on susceptibility to later infestation was demonstrable.

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THE INFLUENCE OF PLANT NUTRITION ON SUSCEPTIBILITY OF PINEAPPLE PLANTS TO MEALYBUG WILT[1, 2]

WALTER CARTER

(Accepted for publication December 30, 1944)

INTRODUCTION

For the past several years experiments have been conducted on the relationship between plant nutrition and susceptibility to mealybug wilt of pineapple plants. The primary object of these tests was to determine whether or not susceptibility could be reduced under field conditions by modifications of the prevailing fertilizer practice. Initially, tests were with plants grown at varying levels of nitrogen, potash, calcium and iron fertilizer, each element being considered in a separate experiment. In later experiments, combinations of nitrogen, potash, iron, and partial sterilization of the soil were studied. All experiments were in the field on a scale sufficiently large to permit statistical analysis of the data and at the same time to afford an unusual opportunity for observations on the etiology of the disease. The latter are recorded in a concurrent paper.

TESTS WITH NITROGEN

Test 1

In this test the fertilizer schedule called for four variants of the nitrogen supplied, namely, no nitrogen, 100, 500, and 1,000 lb. per acre, applied in aliquots of 100 lb. each, beginning in November, shortly after the field was planted, and continuing, for the 500- and 1,000-lb. plots, bimonthly and monthly, respectively. All the plots received superphosphate and potassium sulphate uniformly. Plots were arranged so that three periods of infestation could be used and for each period there were six replications of each treatment. On account of the fertilizer schedule, there was actually a different N variable for each infestation period, the final aliquot for all the 500- and 1,000-lb. plots being applied after the last infestation.

A standard method of mealybug infestation was used. Mealybug-infested pineapple plants were collected from fields showing wilt and the medium-sized mealybugs were taken from them in large numbers. From this mass of mealybugs, aliquots of 50 were put into separate vials, usually in the late afternoon, and used to infest individual plants the following morning. Infestations were sprayed out after two weeks had elapsed. Following infestation, the plants were kept under observation until they fruited the following year. There was no relationship between N level and wilt incidence. About 70 per cent of the plants wilted when infested at approxi-

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[2] Acknowledgment is made to Dr. H. E. Clark and members of his staff of the Physiology-Soils Department and to Mr. K. Ito, Assistant Entomologist, for their cooperative assistance in this study.

mately three months old, but there was a sharp drop in the percentage wilted in the plots in which the plants were infested at five months and seven months of age, confirming previous evidence that susceptibility decreases with age of plant.

Test 2

In this test, nitrogen levels of 0, 500, and 1,000 lb. to the acre were used with a uniform application of 300 lb. per acre each of phosphorus and potassium. There were 28 plots of 20 plants each and all the plots were planted with slips of a single clone on October 27, 1938. Fifty mealybugs per plant were applied on April 20–22, 1939, approximately six months after planting, and were allowed to feed for approximately two weeks. Final wilt readings were taken September 10, 1939. Plots were kept under observation until fruit harvest and no change in the incidence of wilt occurred. In this test the average percentages of wilted plants were 34, 32, and 19, the latter being in the 1,000-lb. N plots. This difference was significant at odds of 19 to 1.

Test 3

On September 26, 1940, following fruit harvest of plots of test 2, the first ratoon suckers on the mother plants that had failed to wilt when infested six months after planting, were reinfested. The sucker production in the no-nitrogen plots was small, as could be expected, but every well-developed sucker was infested with mealybugs. In this infestation a different technique was used, in that a piece of the rind from heavily infested fruits was dropped into the center of each plant. About three weeks later the mealybug colonies established on the plants were sprayed out. This meant a considerably heavier infestation than the standard 50-mealybug lots used previously, but the method permits the infestation of the plants in a more natural manner without excessive handling of the insects and with the reduced labor involved makes it possible to infest on a very much larger scale. The number of mealybugs applied by the fruit rind method is always in excess of that shown by previous experiments to give high percentages of wilted plants, although there is considerable variation between individual pieces.[3] The result from counts of the mealybugs on 15 pieces of fruit rind taken at random showed the number of gravid females to vary from 1 to 22, the medium-sized bugs from 24 to 102 and the crawlers from 140 to 690, with a minimum number of all classes of 165 on a single piece of fruit rind.

TEST WITH POTASH

Test 1

This test was in the same field and in the beds contiguous to those used for nitrogen test 2. All the plots were planted to the same clone, and received uniform applications of nitrogen and phosphorus. Potash was varied

[3] Carter, Walter. The toxic dose of mealybug wilt of pineapple. Phytopath. 27: 971–981. 1937.

at 0, 300, and 600 lb. per acre. The plots were planted October 27, 1938, and each plant infested with 50 mealybugs March 29, 1939, at which time the potash applications had been completed. In August, 1939, all the plants which had failed to show symptoms were reinfested with a second dose of 50 mealybugs. Plant analyses made at that time revealed material differences between treatments, with respect to potash and nitrate N, both of which increased with increased amounts of potash applied. Wilt in the 300-lb. plots showed a significant increase (odds of 19 to 1) over that in the no-potash and 600-lb. plots.

Test 2

This experiment corresponds to test 3 of the nitrogen experiment in which the suckers growing on the previously infested plants were infested grossly by the fruit rind method. Wilt incidence was uniformly high as in nitrogen test 3, which was concurrent.

TESTS WITH CALCIUM

In these tests, 25 cc. of a 5 per cent solution of calcium chloride were applied to the axils of the base leaves of field grown plants weekly, fortnightly, and monthly. Other fertilizer applications were uniform. These applications were made from February to March 22, 1939, on plants planted late in October of the previous year. All the plants were infested with 50 mealybugs per plant on April 13, 14, and 15 but no significant differences in wilt incidence resulted.

TESTS WITH IRON

These tests were run parallel with the nitrogen, potash, and calcium experiments. The plants were fertilized uniformly but there were three variations in the iron treatment: (1) no iron spray, (2) iron spray every two weeks, and (3) iron spray twice weekly. A 4 per cent solution of ferrous sulphate was applied in a fine mist shortly after planting in October, 1938, and continued until the end of the experiment. Fifty mealybugs per plant were applied April 26, 27, and 28. Iron deficiency was becoming evident in the no-iron plots at the time infestations were made, and pronounced differences developed thereafter. The plots sprayed with iron twice a week, an excessive quantity according to plantation practices, nevertheless responded to the increased iron supply. There were, therefore, three quite clearly defined differences in greenness of the plants when the mealybugs were applied and the differences were maintained throughout the experiment, but no significant differences in wilt incidence occurred. Infestations of the young first ratoon suckers in both the calcium and iron plots were made at the same time as were those in the nitrogen and potash experiments with no significant differences resulting.

TESTS WITH NITROGEN, POTASH, IRON, AND PARTIAL STERILIZATION OF THE SOIL IN COMBINATION

These plots were arranged in a factorial manner to permit more adequate

statistical analysis of the results. Furthermore, the experiments were on such scale that from 7,000 to 9,000 plants were involved in each test. Table 1 applies to all the experiments in this series, and shows the plot arrangement.

TABLE 1.—*Experiments on the effect of nitrogen, potash, iron and soil sterilization on susceptibility. Diagram showing factorial arrangement of plots*[a]

Plot	Low Fe			Plot	High Fe			Plot	High Fe			Plot	Low Fe		
1	N1	K2	S1	17	N1	K2	S1	33	N1	K2	S2	49	N1	K2	S1
2	2	2	2	18	1	1	2	34	1	1	1	50	2	1	1
3	2	1	1	19	2	1	1	35	2	1	2	51	2	2	2
4	1	1	2	20	2	2	2	36	2	2	1	52	1	1	2
5	1	1	1	21	2	1	2	37	1	2	1	53	2	2	1
6	2	1	2	22	1	2	2	38	2	1	1	54	2	1	2
7	2	2	1	23	2	2	1	39	1	1	2	55	1	1	1
8	1	2	2	24	1	1	1	40	2	2	2	56	1	2	2
Plot	High Fe			Plot	Low Fe			Plot	Low Fe			Plot	High Fe		
9	2	1	2	25	2	2	2	41	1	1	1	57	1	1	2
10	1	1	1	26	2	1	1	42	2	2	1	58	1	2	1
11	2	2	1	27	1	1	2	43	2	1	2	59	2	1	1
12	1	2	2	28	1	2	1	44	1	2	2	60	2	2	2
13	2	2	2	29	1	1	1	45	1	1	2	61	2	1	2
14	1	1	2	30	2	2	1	46	1	2	1	62	1	2	2
15	1	2	1	31	1	2	2	47	2	2	2	63	1	1	1
16	2	1	1	32	2	1	2	48	2	1	1	64	2	2	1

[a] Legend: N1 = 100 lb. nitrogen per acre; N2 = 500 lb. nitrogen per acre; K1 = no potash; K2 = 300 lb. potash per acre; Fe1 = 4 per cent $FeSO_4$ applied as a fine mist monthly; Fe2 = 4 per cent $FeSO_4$ applied twice weekly; S1 = no sterilization of the soil; S2 = 150 lb. per acre of chloropicrin injected into the soil before planting.

Test 1

This experiment was planted early in October of 1939. An infestation was made February 20 and 21, 1940. Infested immature fruits were used and a section of the rind placed in the heart of each plant, as in nitrogen test 3. On February 28, 1940, establishment of vigorous mealybug colonies seemed to be general, and one month after infestation the plots were sprayed with an oil emulsion to eliminate the mealybugs. The final wilt readings on these plots were made on July 24, 1940, and the data are summarized in table 2.

Test 2

This experiment was an exact duplicate of the first and was in exactly the same area with only a short 3-month intercycle period between clearing the plots of the plants from test 1 and planting those of test 2. The purpose of this arrangement was to determine whether the position of wilted plants would remain the same as in test 1. The plants were planted October 2, 1940, and infested with mealybugs on February 25, 1941. Wilt readings were completed on July 30, 1941, and are summarized with those of test 1 in table 2, for comparison with results from test 1.

Test 2a

This test was accomplished by reinfesting one half the plots of test 2, on July 30, 1941, when the wilt readings for test 2 were completed, and the plants which had shown wilt were recovering. The results of this reinfestation, shown in tables 3 and 4, reveal a curious significance in the differences between certain of the treatments but only in the case of those plants which had recovered from severe wilt. Plants in the low N plots wilted a second time in numbers significantly greater than in the high N plots, and, to a lesser degree, significantly greater in the no-chloropicrin plots than in those treated with chloropicrin (Table 4).

TABLE 2.—*Factorial tests 1 and 2. Experiments on the effect of nitrogen, potash, iron, and soil sterilization on susceptibility, the plots being factorially arranged. Table showing number of plants wilted in tests 1 and 2. Plants infested when five months old. Each plot included approximately 138 plants with four replications of each treatment*

Treatment				Test 1: Infested 1940						Test 2: Infested 1941					
				Plants wilted						Plants wilted					
				Replications				Total	Per cent	Replications				Total	Per cent
N1	K2	S1	F1	1	2	3	4			1	2	3	4		
2	2	2	1	102	96	82	113	393	71	106	95	129	96	426	77
2	1	1	1	93	61	91	88	333	60	78	78	116	102	374	68
1	1	2	1	95	106	115	88	404	73	123	101	109	100	433	78
1	1	1	1	63	84	98	67	312	57	97	99	128	123	447	81
2	1	2	1	74	118	68	72	332	60	88	109	82	82	361	65
2	2	1	1	78	69	106	77	330	60	109	106	107	107	429	78
1	2	2	1	104	96	114	93	407	74	115	131	78	96	420	76
2	1	2	2	91	70	116	70	347	63	96	130	105	68	399	72
1	1	1	2	68	82	118	92	360	65	88	109	119	129	445	81
2	2	1	2	100	89	72	78	339	61	92	79	97	106	374	68
1	2	2	2	93	94	88	75	350	63	131	92	132	89	444	80
2	2	2	2	112	73	89	65	339	61	105	99	111	129	445	80
1	1	2	2	101	72	72	99	344	62	122	91	55	116	384	70
1	2	1	2	73	112	73	88	346	63	126	80	75	68	349	63
2	1	1	2	65	84	76	99	324	58	122	77	113	62	374	68
				99	92	60	116	367	66	111	124	94	104	433	78

TABLE 3.—*Results of reinfesting plants in factorial test 2 which had recovered from severe wilt, initial symptoms only, or had remained symptom-free following the first infestation*

Treatment group	No. of plants reinfested in each class			Percentage plants wilted in each class		
	Symptom-free	Initial stages only	Severe wilt	Symptom-free	Initial stages only	Severe wilt
All	1,129	278	410	34.7	33.4	30.0
Low N	651	113	232	33.5	38.1	35.8
High N	478	165	178	36.4	30.3	22.5
No chloropicrin	530	161	177	34.1	34.6	35.5
Chloropicrin	599	117	233	35.2	32.4	25.7
Low K	554	93	216	34.4	37.6	26.8
High K	575	185	194	34.9	31.3	33.5
Low Fe	552	176	232	37.1	34.0	26.7
High Fe	577	102	178	32.4	32.3	34.2

TABLE 4.—*Statistical treatment of data from table 3. Underlined figures are significant*

Plant types reinfested	χ^2 values for the different treatments				χ^2 values from table	
	Low vs. high			Chloropicrin + vs. −	P = 0.05	P = 0.01
	N	K	Fe			
Healthy	1.033	0.029	2.783	0.143	3.841	6.635
Initial stages only	1.810	1.097	0.088	0.086	3.841	6.635
Severe wilt	8.489	2.154	2.731	4.640	3.841	6.635

Test 3

This test was laid out in precisely the same manner as tests 1 and 2, the only difference being that the plots were not so large, each plot containing about 85 plants instead of about 140. The plants grew without mealybug infestation until they were 9½ months old, when they were infested by the fruit-rind method. By this time, very great differences in growth status had developed throughout the plots. The results of this test are in table 5. The low percentage of plants wilting, when compared with tests 1 and 2 is further confirmation of the reduced susceptibility of older plants.

Test 4

This was a duplicate of test 3 as far as factorial arrangement, size of plots, age of plants, and time of infestation were concerned, except that only

TABLE 5.—*Factorial tests 3 and 4. Experiments on the effect of nitrogen, potash, iron, and soil sterilization on susceptibility, the plots being factorially arranged. Table showing number of plants wilted in test 3. Plants infested when 9½ months old. Each plot included approximately 85 plants with four replications for each treatment in test 3, and 2 replications in test 4*

Treatment				Test 3: Infested 1941						Test 4: Infested 1942			
				Plants wilted						Plants wilted			
				Replications				Total	Per cent	Replications		Total	Per cent
				1	2	3	4			1	2		
N1	K2	S1	F1	5	20	21	6	52	16	24	26	50	29
2	2	2	1	3	8	26	8	45	13	10	11	21	12
2	1	1	1	10	19	22	2	53	16	31	18	49	28
1	1	2	1	29	30	22	32	113	33	20	24	44	25
1	1	1	1	39	25	24	30	118	35	28	46	74	43
2	1	2	1	31	12	32	20	95	28	30	33	63	36
2	2	1	1	13	37	25	24	99	29	40	18	58	33
1	2	2	1	12	36	21	34	103	30	40	37	77	45
2	1	2	2	13	20	6	11	50	14	16	17	33	20
1	1	1	2	19	13	12	34	78	23	41	6	47	27
2	2	1	2	19	33	17	21	90	26	24	13	37	22
1	2	2	2	15	28	6	15	64	19	29	16	45	26
2	2	2	2	21	24	33	12	90	26	12	39	51	30
1	1	2	2	40	12	23	20	95	28	13	17	30	18
1	2	1	2	34	8	18	24	84	25	1	13	14	8
2	1	1	2	13	10	21	22	66	20	18	36	54	32

one-half the total number of plots were infested. Wilt following the infestation in this test is shown along with the data from test 3, in table 5.

DISCUSSION

When plants are infested within three months of planting, differences in growth status due to fertilizer level are apparent but may not have had sufficient opportunity to affect susceptibility significantly. From six months on, however, the variations in fertilizer practice used in these experiments resulted in extreme variation in plant growth. The low-nitrogen plants were small, yellow green, with tough narrow leaves, while at the other extreme were the large, dark green, succulent, wide-leaved plants in the high N plot, especially where the soil was partially sterilized before planting.

In spite of these extremes, no striking relationship exists between plant growth status and susceptibility to wilt. The detailed data were subjected to analysis of variance with the result that significance ($p = .05$ or odds of 19 to 1) was shown in nitrogen test 2 to the extent that wilt was less in the 1,000-lb. N plot than in either the 0- or 500-lb. N plots. In potash test 1, there was significantly more wilt in the 300-lb. plot than in either the 0- or 600-lb. plots. Since other experiments including these same variables failed to show similar results, too much importance cannot be attached to these significances. Variation between numbers of plants wilted in plots of similar treatment are so great that very great differences between treatments would be necessary to show significance. This variation between plots is believed due to the localization of wilt in small areas within the field. This phenomenon is discussed further in the concurrent paper, but it is clear that it has overwhelmed any effect on susceptibility that the fertilizer treatments might have had.

When the four factorial experiments were analyzed, statistical significance was shown in test 3, not between the two iron treatments, but in the interactions between iron and other experimental variables. The factorial experiments were on such an extensive scale that this result cannot be disregarded entirely, even though none of the other experiments showed a similar result; but to account for it, a very delicate balance must be postulated between plant nutrition and photosynthetic activity which in some manner affects susceptibility.

Test 2a, in which plants were infested for a second time, shows the only striking relationship between treatments and wilt incidence. Plants which had had severe wilt symptoms following the first infestation but had recovered in typical fashion, wilted a second time following reinfestation by mealybugs in significantly greater numbers ($p = 0.01$ or odds of 99 to 1) in the low N plots than in the high N plots, and to a lesser significant degree ($p = 0.05$ or odds of 19 to 1) in greater numbers in the no-chloropicrin plots (Tables 3 and 4). Whatever the correct explanation for these results may be, it is clear that plants which were recovering from severe wilt were rendered more susceptible to wilt a second time under conditions adverse to

growth. From the standpoint of mealybug wilt control, the results obtained when young ratoon suckers were infested, make it clear that wide variations in fertilizer application to mother plants failed to affect the extremely susceptible young suckers. Since this period in the life of a pineapple field is the most critical one with respect to increase in mealybug wilt incidence, there is little hope of modifying the severity of mealybug wilt by agronomic methods.

SUMMARY

Pineapple plants grown at various levels of N, P, K, Ca, and Fe develop with extreme variation in growth status, which was accentuated by partial soil sterilization with chloropicrin. These plants were infested with mealybugs and the resulting mealybug wilt recorded, but the differences in fertilizer application failed to affect the susceptibility of the plants to wilt. High N application appeared to reduce susceptibility, but in one test only.

The most significant result was in the reinfestation of plants that were recovering from severe wilt. Adverse growth conditions increased the susceptibility of these plants to a second wilting.

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A CERCOSPORA LEAFSPOT OF CULTIVATED PHYSOSTEGIA[1]

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INTRODUCTION

During August, 1943, a destructive leaf spot of cultivated *Physostegia virginiana* Benth. came to the writer's attention. The disease was so severe and the resulting defoliation so acute that an effort was made to identify the pathogen. After it became apparent that the disease was caused by an undescribed species of *Cercospora,* a study of the life cycle of the pathogen was undertaken. A portion of the results form the basis of this report.

SUSCEPTS AND RANGE

Field observations have been confined to a small area in the vicinity of Chatham, Virginia, where the disease is prevalent. Nothing is known of the general distribution of the disease, nor is the disease apparently known on wild relatives of the species in question.[2] Somewhat closely related species of the mint family in this area show no evidence of susceptibility to the disease.

SYMPTOMATOLOGY

The earliest observed symptoms appeared during the middle to latter part of June on the lowermost leaves. Typical symptoms persist on these lower leaves, scarcely noticeable to the casual observer until the latter part of July, but meanwhile progressing slowly up the plant from leaf to leaf. Very few symptoms have been seen on any portion of the plant other than the foliage, though when the disease becomes epiphytotic during August it is not uncommon to find lesions on the flower bracts.

Lesions vary from one to several centimeters, are at first pale yellow with slightly darker centers, and generally appear first on the upper leaf surface. A typical halo, usually rather consistently associated with such lesions, is absent. As the lesion develops, its color approaches brick-red and finally becomes pale to dark brown. Meanwhile, the lesion spreads rather uniformly over the leaf surface, finally becoming slightly depressed and dry (Fig. 1, A). On the lower leaf surface, a generalized pale color soon marks the limits of the lesion during early developmental stages. Later, color changes occur within the lesions, but they are never so intense as those on the upper leaf surface, nor are the limits of the lesions ever so well defined (Fig. 1, B). Through coalescence of lesions, most of the surface of affected leaves is ultimately involved; the leaves may curl or there may be more or less complete defoliation.

[1] Published with the approval of the Director, Virginia Agricultural Experiment Station, as Scientific Paper No. 123 from the Section of Botany and Plant Pathology.

[2] After this report was submitted for publication, correspondence and exchange of specimens with Dr. B. B. Higgins established the presence of this disease in the vicinity of Griffin, Georgia, where it was destructive in 1941.

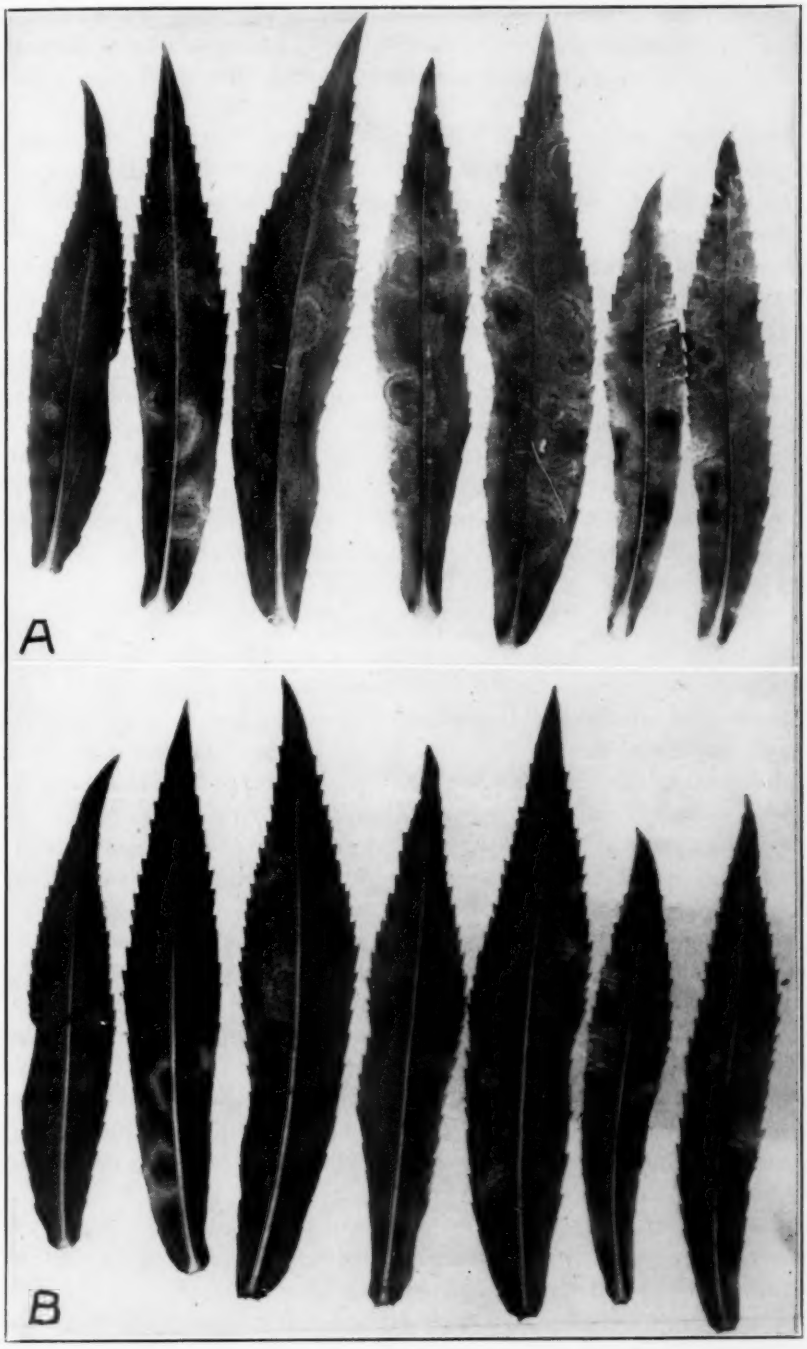

FIG. 1. Photographs showing a graduated series of symptoms of Cercospora leaf spot of leaves of cultivated *Physostegia virginiana* Benth. (A) Upper leaf surface; (B) lower leaf surface. The semblance of a halo about certain lesions is due to the photographic filter. (Original photographs × ½.)

Conidiophore stromata appear as discrete, tawny tufts within the older lesions. Normally, sporulation is confined to the upper surface of the lesions. With age and at high humidity sporulation often becomes amphigenous.

ETIOLOGY

Being unable to determine the specific identity of the pathogen with the literature at hand, the author sent specimens to Professors F. A. Wolf and Charles Chupp, of Duke University and Cornell University, respectively. Professor Wolf reported that no species of *Cercospora* on *Physostegia,* nor on its synonym, *Dracocephalum,* was listed in the literature available to him and that the species appeared to be undescribed. Professor Chupp stated that the species was new according to his keys and that it did not resemble any other of the known forms on the Labiatiae. He also stated that he was filing the specimens in his herbarium as *Cercospora Physostegiae* W. A. Jenkins, awaiting my description of the species. Because we now know that the pathogen possesses a normal and typical life cycle, including spermogonia and perithecia, typical of species of *Mycosphaerella* heretofore described (1, 2, 3, 4, 5), it will hereafter be referred to as *Mycosphaerella Physostegiae* n. sp.

DEVELOPMENTAL MORPHOLOGY

Results of inoculations under controlled conditions indicate that infection may occur through either leaf surface, though more uniformly through the lower one, and that penetrations occur through stomata or directly through epidermal walls. Infection hyphae appear to be intercellular at first, but soon an intracellular relationship is established. Haustoria, in the accepted sense of the term, were not seen, though the characteristic coiling of the hyphae within parasitized cells, approximating a loose stromatic formation, might readily be assumed to perform haustorial functions. Conidiophores originate from subcuticular or intraepidermal hyphae and begin the production of conidia soon after emergence, long before basal anastomosis with attendant stroma formation is evident (Fig. 2, A). With age they assume the characteristics of typical conidiophores of *Cercospora.*

The conidia, en masse, appear bluish-grey; singly they are hyaline to subhyaline. They are slender, more or less curved, blunt on the basal end, tapering to subacute on the distal end, with a tendency toward two oil droplets within each cell of the older spores, 1–6 septate, and $17–112 \times 2.28–6.08\ \mu$ (Fig. 2, B). Under average field conditions, the spores average $48.5 \times 4.24\ \mu$, but those produced at high humidity may be as long as $112\ \mu$. Length and septation of conidia, as with other species of *Cercospora* studied, vary with relative humidity in the environment during their formation, but width remains relatively constant. The spores germinate readily in from 3 to 8 hours on tap-water agar when the moisture and oxygen balance are favorable. Germ tubes emerge from terminal cells at either or both ends of the spore, and often from other cells, as well (Fig. 2, C).

During late September and early October, the saprophytic cyclic develop-

ment is initiated within the leaves that have fallen from the plants. Some evidence of such development may also be noted on foliage still attached, but it is evident that such leaves are essentially senescent, so that one might readily assume the relationship to be a saprophytic one. Spermogonia and perithecia are initiated concurrently, either within conidiophore bases or independently of such structures. In the latter instances, spermogonial and

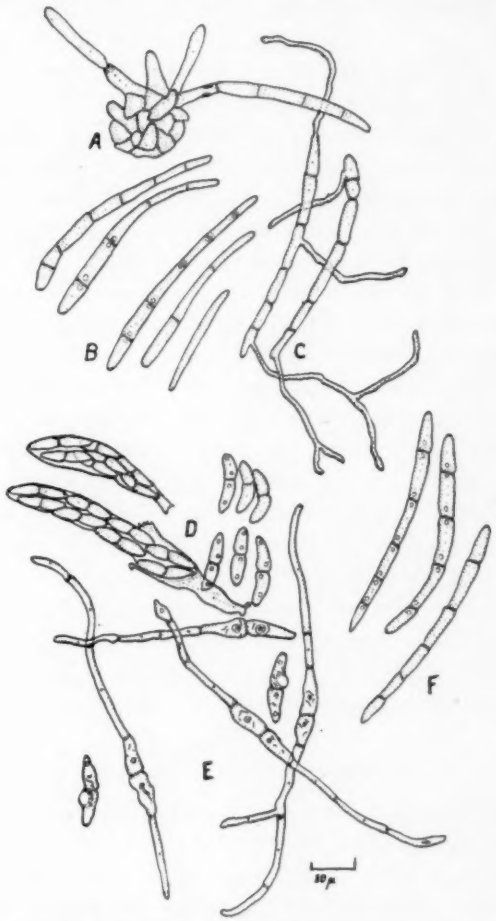

FIG. 2. Spore forms of *Mycosphaerella Physostegiae* n. sp. A. Young conidial stroma before development of a pronounced basal stroma. B, C, F. Conidia: B, from field material; C, showing method of germination; D, from ascospore culture on potato-dextrose agar. D, E. Asci and ascospores: D, an ascus intact, a mature ascus with ruptured outer membrane, and recently discharged ascospores; E, germinating ascospores. (All sketches drawn to scale, original with aid of a camera lucida, ×1,200 dia.)

perithecial fundaments originate from subcuticular, intraepidermal, or rarely, from subepidermal stroma. Owing, perhaps, to fluctuating climatic conditions, it is not unusual to find fertile conidiophores developing from the exterior surfaces of both spermogonia and perithecia.

Very young spermogonia and perithecial fundaments are indistinguish-

able one from the other, but with limited development they may be readily separated on the basis of content. As with other species of *Mycosphaerella* studied critically (1, 2, 3), the perithecial fundaments soon produce one to several archicarps, while the spermogonia develop spermatial mother cells.

The spermogonia originate about the periphery of old lesions or within them and are about equally abundant on both leaf surfaces. The details of development are essentially identical with those reported for other species of *Mycosphaerella*. At maturity, the spermatia occur in groups of 3 or 4 within the spermatiferous cells and are liberated through sterigma-like processes from them. Mature spermogonia are 27–60 × 27–56 μ. Mature spermatia are rod-shaped, uninucleate, hyaline, and 1.5–4 × 0.5–0.8 μ.

Perithecial fundaments originate concurrently with the spermogonia. Their distribution is also the same, but they soon produce one to several deep-staining archicarps, each with an elongate trichogyne and an enlarged, uninucleate basal cell. Cytological details of perithecial development thus far observed differ in no important respects from those already published for other species of the genus (1, 2, 3). The perithecia are rostrate when young and at maturity are 47–95 × 40–75 μ.

The perithecia of *Mycosphaerella Physostegiae* develop slowly as compared with several other known species, mature spores in the field having first been found on June 9, 1944. Sectioned material confirmed the week of June 9 as being the time of earliest maturation of spores. This delay in perithecial maturity is occasioned by the very slow development of the ascogonia following spermatization. The asci do not mature at one and the same time within a given perithecium so that perithecia function as the source of primary inoculum over a considerable period, depending on the frequency of moisture. In addition, conidia are produced abundantly from the surfaces of both sterile and fertile stromatic masses, beginning as early as May 12 in 1944, and continuing on concurrently with ascospore maturation and discharge.

DEVELOPMENT IN CULTURE

Conidial isolates were obtained by the streak method on hard tap-water agar, while ascospore isolates were obtained by causing the ascospores to discharge upward and cling to the surface of tap-water agar in inverted Petri dishes. Mature spores were measured in crushed mounts and soon after their discharge into the Petri plates. Under such conditions the ascospores are hyaline to subhyaline, guttulate when young, straight to slightly curved, bicellular, and 11.6–17.8 × 2–3.8 μ. The asci are bitunicate and the ascospores become biseriately arranged prior to discharge (Fig. 2, D). Because of its structure and the dynamics attendant on its function, a mature ascus is difficult to measure for length; nor, in the writer's opinion, is such a measurement of taxonomic value (Fig. 2, D).

Spores of both types germinate readily within 3 to 8 hours and produce visible growth within 3 days (Fig. 2, C–E). Cultures from conidia and ascospores on potato-dextrose agar are identical in all measurable respects.

When first visible, the mycelium is pale grey to almost white, but the portion of the colony adjacent to the medium finally becomes deep olive to almost black. The colonies are pulvinate from the beginning and after 5 to 6 days indications of a stroma appear adjacent to the surface of the medium, particularly in isolates from ascospores. Isolates from both sources produce conidia abundantly within 3 to 5 days and the conidia are indistinguishable from each other and from those produced under similar conditions of temperature and moisture on field material (Fig. 2, B, F). Stromatic masses were produced in old cultures from both sources, but recognizable spermogonia and perithecia were not observed in any of the cultures.

Both conidia and ascospores were used as inoculum. The conidia were obtained from fresh field material as well as from conidial and ascospore isolates. In all cases resulting infections were identical and occurred within 10 to 21 days following inoculation.

The spore forms discussed constitute a pleomorphic cycle of one and the same organism.

TAXONOMY

The form and development of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the bicellular, hyaline spores are characteristic of the genus *Mycosphaerella* Johans. The fungus is designated *Mycosphaerella Physostegiae* n. sp., with the following diagnosis.

Mycosphaerella Physostegiae n. sp.

Syn: *Cercospora Physostegiae* W. A. Jenkins (In Herb.)

Perithecia numerous, mostly in lesions, amphigenous, partly embedded in host tissue, erumpent, ovate to nearly globose, beaked prior to maturity, 47–95 × 40–75 μ, black, ostiolum papillate when mature; asci club-shape, short stipitate, fasciculate, 36–40 × 10–20 μ, aparaphysate, bitunicate, eight-spored; spores biseriate to imperfectly uniseriate in the ascus, bicellular, straight to slightly curved, hyaline to subhyaline, guttulate, 11.6–17.8 × 2–3.8 μ.

Hab. In overwintered lesions produced by the conidial stage on leaves of *Physostegia virginiana* Benth. (cultivated), Chatham, Virginia, maturing during early June.

Spermogonia: Numerous, in and along the margins of lesions produced by the conidial stage, ovate to globose, black, amphigenous, embedded in leaf tissue but later erumpent, ostiolate, 27–60 × 27–56 μ; spermatia small, rod-shaped, hyaline, 1.5–4 × 0.5–0.8 μ, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

Hab. On recently fallen leaves, maturing from October through February.

Conidial stage: Lesions circular to indefinite, often confluent, varying from 1 mm. to several cm., pale to dark brown, confined to foliage; conidiophores becoming amphigenous with age, base becoming stromatic; fasciculate, geniculate, pigmented at base, continuous to one- to several-septate, mostly short; conidia hyaline to subhyaline, slender, cylindrical, club-shaped, blunt on basal end, tapering to subacute on distal end, curved, 17–112 × 2.28–6.08 μ (mostly 48–60 × 3.8–4.2 μ), 1–6 septate, guttulate, length and septation influenced by humidity.

Hab. Parasitic on leaves of cultivated *Physostegia virginiana* Benth., causing leaf spots and contributing to premature defoliation.

Peritheciis dense aggregatis, plerumque in maculis, amphigenis, semi-immersis, punctiformibus, ovatis vel globatis, rostratis cum immaturis, 47–95 × 40–75 μ, nigris, ostiolis papillato praeditis; ascis cylindraceis clavatis, brevisme stipitatis, fasciculatis, aparaphysatis, bitunicatis, octosporis, 36–40 × 10–20 μ; sporiidis biseriatis vel sub-uniseriatis, bicellularibus, vix curvatis, hyalinus vel sub-hyalinis, guttulatis, 11.6–17.8 × 2–3.8 μ.

Hab. in foliis dejectis *Physostegia virginianae*, Chatham, Virginia.

Spermogoniis autumno efformatis, dense aggregatis, plerumque in maculis et marginatis, ovatis vel globatis, nigris, amphigenis, innatoerumpentibus, punctiformibus, 27–60 × 27–56 μ; spermatiis bacillaribus, hyalinis, 1.5–4 × 0.5–0.8 μ.

Hab. in foliis dejectis *Physostegia virginianae*.

Statu conidico in maculis circularibus v. irregularibus, saepe confluentibus, ocraceis v. rubro-ferrugineis, foliis efformato; hyphis fertilibus amphigenis, a stromate orientibus, fascilatis, geniculatis, hyalinis v. dilute olivaceis-grieseis, continuis v. pluriseptatis, brevisme; conidiis hyalinis v. sub-hyalinis, attenuatis cylindraceis obclavatis v. subclavatis, curvulis, 17–112 × 2.28–6.08 μ (plerumque 48–60 × 3.8–4.2 μ), 1–6 septatis, guttulatis.

Hab. in foliis vivis *Physostegia virginianae*.

For the convenience of plant pathologists and mycologists, materials have been deposited in the following herbaria: Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture; Plant Pathology Department, Cornell University, Ithaca, N. Y., the Herbarium of the Georgia Experiment Station, Experiment, Georgia.

CONTROL

Results from a single season's work indicate that Cercospora leaf spot of cultivated *Physostegia virginiana* Benth. may be rather effectively controlled by simply removing the fallen leaves from beneath the planting any time before mid-April of the following year. Since the plantings in Virginia are propagated by rhizomes, it would be impractical to turn under the fallen leaves. The late maturity of the perithecia greatly simplifies removal and destruction of the fallen leaves, preferably by burning, as a control measure.

SUMMARY

The symptomatology and etiology of a destructive leaf spot of cultivated *Physostegia virginiana* Benth. have been studied over a complete season. Symptoms first appear during June and July on the lowermost leaves, but the disease does not become epiphytotic until August. The lesions appear pale yellow at first with dark centers, but develop through shades of brick-red to a final pale to dark brown and then enlarge until often most of the leaf surface is involved.

The pathogen, heretofore undescribed in any stage of development, produced a hyphomycetous conidial stage characteristic of the form genus *Cercospora*, also spermogonia and perithecia. The perfect stage is herein described as *Mycosphaerella Physostegiae* n. sp.

A single season's observations indicate that Cercospora leaf spot of cultivated *Physostegia virginiana* Benth. may be controlled by removing and burning the cast leaves any time between leaf fall and mid-April of the following season.

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VARIETAL RESISTANCE AND SUSCEPTIBILITY OF WHEAT TO FLAG SMUT (UROCYSTIS TRITICI KÖERN.). IV. FURTHER STUDIES ON PHYSIOLOGIC SPECIALIZATION IN UROCYSTIS TRITICI KÖERN.[1]

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INTRODUCTION

In an earlier paper (4), the senior writer and his co-workers reported 5 physiologic races of *Urocystis tritici* Köern. from China. Since the publication of that paper, additional collections of the smut from various parts of China have been tested and 7 new races have been described and numbered, thus bringing the number of known races to 12.

MATERIAL AND METHODS

In addition to the 5 physiologic races of *Urocystis tritici* reported in 1936 (4), 37 smut collections were obtained from common wheat (*Triticum vulgare*), and one collection on a poulard wheat (*T. turgidum*) was obtained and used in 1942–43.

For differential hosts, 17 varieties of wheat including Nanking Nos. 716, 793, 795, and 799, which had been used in the previous studies, were chosen. They are outstanding for certain characters and representative of some wheat growing regions of China. In the final analysis of the results, however, only 5 of them have been selected as differential hosts: Nanking No. 716, developed by the Department of Plant Pathology, Nanking University, for its high yield and flag smut resistance (3); Grassland, a high-yielding variety in Kweichow, originated at Kweichow Provincial Agricultural Experiment Station; Ngochen, reported to have originated from the commercial wheat grown in Ngochen; Tsing Hua No. 1932, originated by selection from commercial wheat in Yunnan; and Tsing Hua No. 559, a poulard wheat (*Triticum turgidum*), commonly grown in Central South China. With the exception of the last one, all are common wheat (*T. vulgare*).

All seed was disinfected in a formaldehyde solution (1:320) for 1 hour and washed and dried before sowing. A measured quantity of spores was shaken with the seed until the seed was completely covered with spores. A complete series of wheat hosts was so inoculated for each collection of smut. The inoculated seed in each year's test was planted at the rate of 3 grams to the 3-foot row. Each plot consisted of three 3-foot rows for each variety,

[1] Paper No. 21 from the Division of Plant Pathology, The Institute of Agricultural Research, National Tsing Hua University, Kunming, Yunnan, China.

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Phytopathology extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

systematically distributed and replicated in 4 series. The check plots of uninoculated seed of each variety so treated produced smut-free plants. The smut percentages obtained were based on counts of the total number of culms per plot. As there was very little difference in the percentages of smut in the replicated plots, only the averages are recorded.

In the analysis of data, three infection classes were arbitrarily established: 0–5 per cent infection = Resistant class (R); 5.1–20 per cent infection = Intermediate class (I); and 20.1–100 per cent infection = Susceptible class (S).

RESULTS

The experiments were at Kunming, Yunnan, China, from 1939 to 1943, inclusive. The 5 physiologic races previously reported, together with 37 additional collections of the smut, were tested on 17 varieties of common wheat. Twenty-one of these 37 smut collections produced consistent results and have been identified. The rest of them are reserved for further studies. Of the 17 varieties of common wheat, 4, namely, T. H. 1932, N. 716, Ngochen, and Grassland, were retained as differential hosts. The others, on which results had been inconsistent, were discarded. On the basis of the reaction of these 4 varieties of wheat, it was possible to differentiate the smut collections into 11 physiologic races. Five of them had been previously identified and the remaining 6 are recorded as new races. The annual and average percentages of smut produced by each of the smut collections, in each of the 4 years tested, are given in table 1.

There are sufficiently great differences in the virulence of the collections of smut to justify the conclusion that they are different physiologic races. Races 4 and 5 are differentiated from the rest by the intermediate reaction of N. 716. T. H. No. 1932 is resistant to race 4 but is intermediate in its reaction to race 5, which is the only race that can infect all of the differential hosts. Races 10 and 11 are distinct from other races by their virulence on Grassland and are differentiated by the difference in reaction of Ngochen and T. H. 1932 to them. The identity of race 2 is based on the susceptibility of T. H. No. 1932 to it, that of race 7 on the susceptibility of Ngochen. Race 6 is the only race to which all four differential hosts are resistant. It is similar to races 1 and 9.

The variety T. H. No. 3929 was originally selected as the susceptible check. The high percentages of smut produced on it indicate that the environmental conditions of the tests were favorable for smut infection. This variety is completely susceptible to the 11 physiologic races (Table 1).

In the course of breeding for flag smut resistance in both the common and poulard wheats, about 300 selections of the latter were inoculated with a mixed inoculum of the smut each year since the fall of 1938. In 5 years, none of these poulard wheat selections had ever been smutted. It was thus once believed that the poulard wheat might be immune from the disease. However, in the spring of 1942, an additional collection of flag smut from a commercial field of poulard wheat in Chengkung, Yunnan, was used to in-

TABLE 1.—*Average percentages[a] of flag smut obtained in 5 varieties of common wheat inoculated separately with 21 collections[b] and grouped into 11 physiologic races of Urocystis tritici at Kunming, Yunnan, China, 1940–1943*

Host	Year	Race 1, Collection No.—					Race 2, Collection No.—					Race 3, Collection No.—				
		la	5	6	10	19	2a	11	12	20	21	3a	13	14	16	18
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
T. H. No. 1932	1940	2.1	1.4	2.4	.....	.....	22.6	.....	.....	.....	.....	0.0	.....	.....	.....	.....
	1941	0.0	0.0	0.0	5.6	3.2	18.3	11.3	21.1	22.9	19.1	3.4	2.6	3.1	2.3	0.0
	1942	0.0	0.4	0.5	0.0	0.5	17.5	39.3	43.9	34.1	41.5	2.1	0.9	1.8	4.3	0.0
	1943	0.0	0.0	2.0	2.3	0.8	26.1	18.4	13.5	25.7	24.2	1.7	0.0	0.5	0.6	0.3
	Av.	0.5	0.5	1.2	2.6	1.5	21.1	23.1	26.2	27.6	28.2	1.8	1.2	1.8	2.3	0.1
N. 716	1940	0.0	0.0	0.0	.....	.....	0.6	.....	.....	.....	.....	2.2	.....	.....	.....	.....
	1941	0.0	0.0	0.0	0.0	0.0	1.1	0.3	3.1	0.5	5.4	0.6	3.6	3.7	0.0	0.0
	1942	0.0	2.3	0.0	0.3	0.0	1.4	1.6	7.8	0.0	5.2	3.4	7.4	8.1	2.7	2.1
	1943	3.2	0.7	0.0	0.0	0.0	0.0	0.6	0.0	4.9	1.7	1.5	1.8	2.0	4.1	0.0
	Av.	0.8	0.8	0.0	0.1	0.0	0.8	0.8	3.6	1.8	4.1	1.9	4.3	4.6	2.3	0.7
Ngochen	1940	6.3	0.0	5.2	.....	.....	1.2	.....	.....	.....	.....	10.2	.....	.....	.....	.....
	1941	0.4	0.0	11.4	5.6	7.3	0.3	0.0	0.5	7.1	2.6	6.7	8.2	7.0	3.5	4.8
	1942	9.1	15.7	1.7	9.7	4.1	0.6	9.1	2.9	4.2	7.7	13.3	13.6	14.5	7.0	9.1
	1943	7.8	6.3	3.7	0.3	4.0	1.9	4.0	0.1	3.0	1.9	4.9	7.3	13.4	4.9	7.6
	Av.	5.9	5.5	5.5	5.2	5.1	1.0	3.4	1.1	4.8	4.1	8.8	9.7	11.6	5.1	6.7
Grassland	1940	3.1	4.4	0.0	.....	.....	0.7	.....	.....	.....	.....	9.6	.....	.....	.....	.....
	1941	0.0	3.7	0.0	2.7	0.0	2.4	2.9	0.3	8.1	1.8	8.3	8.2	7.4	7.3	5.6
	1942	3.4	9.2	0.0	4.0	2.2	4.6	4.0	1.4	0.7	6.1	2.5	17.1	20.6	9.2	3.8
	1943	1.5	1.0	0.5	0.0	0.0	1.9	1.0	0.0	1.3	6.0	4.6	19.7	16.0	16.7	6.3
	Av.	2.0	4.6	0.1	2.2	0.7	2.4	2.6	0.1	3.4	4.6	6.4	15.0	14.1	11.1	5.2
T. H. No. 3929	1940	18.1	52.7	31.5	.....	.....	37.2	.....	.....	.....	.....	33.7	.....	.....	.....	.....
	1941	61.3	38.7	25.8	36.5	47.3	48.6	48.9	33.7	52.9	42.1	28.0	29.7	55.1	29.6	18.5
	1942	52.4	71.6	68.1	25.7	62.2	41.9	73.3	75.8	57.1	63.0	41.3	50.9	43.0	51.3	29.5
	1943	31.4	68.0	67.1	42.8	60.4	49.3	66.2	64.2	55.8	48.5	22.6	78.2	67.0	43.3	32.4
	Av.	40.8	57.8	48.1	31.9	56.8	44.2	69.6	57.9	57.4	51.1	31.4	52.9	55.1	48.1	26.8

TABLE 1.—(*Continued*)

Host	Year	Race 4, Collection No.—	Race 5, Collection No.—	Race 6, Collection No.—	Race 7, Collection No.—	Race 8, Collection No.—	Race 9, Collection No.—	Race 10, Collection No.—	Race 11, Collection No.—
		4	7	8	9	17	1	15	21
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
T. H. No. 1932	1940	.....	.....	.....	.....	.....	.....	.....	.....
	1941	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1942	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1943	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Av.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N. 716	1940	.....	.....	.....	.....	.....	.....	.....	.....
	1941	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1942	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1943	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Av.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ngochen	1940	.....	.....	.....	.....	.....	.....	.....	.....
	1941	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1942	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1943	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Av.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Grassland	1940	.....	.....	.....	.....	.....	.....	.....	.....
	1941	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1942	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1943	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Av.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T. H. No. 3929	1940	.....	.....	.....	.....	.....	.....	.....	.....
	1941	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1942	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1943	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Av.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 1.—(*Continued*)

Host	Year	Race 4, Coll. No. —		Race 5, Coll. No. —	Race 6, Coll. No. —		Race 7, Coll. No. —	Race 8, Coll. No. —	Race 9, Coll. No. —	Race 10, Coll. No. —		Race 11, Coll. No. —
		4a	2	5a	8	17	4	1	3	9	15	7
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
T. H. No. 1932	1940	0.0	1.1	9.9	0.0	.....	2.9	7.2	0.0	.....	.....	3.8
	1941	1.7	0.0	11.8	0.0	3.4	0.7	13.6	0.0	5.2	3.2	1.5
	1942	0.0	3.4	14.7	0.0	0.0	3.3	22.7	0.0	5.9	5.0	9.9
	1943	0.9	1.4	6.5	0.0	1.5	0.3	9.8	0.3	0.0	5.6	5.7
	Av.	0.7	1.5	10.7	0.0	1.6	1.8	13.3	0.1	3.7	4.6	5.2
N. 716	1940	7.6	5.5	14.3	0.0	.....	1.5	0.0	0.0	.....	.....	2.8
	1941	11.3	10.1	17.5	0.0	0.0	0.0	0.0	0.0	6.8	5.5	6.0
	1942	8.8	9.2	8.8	1.4	1.4	3.1	3.2	0.4	5.3	3.9	6.5
	1943	10.1	7.4	10.1	0.6	0.0	0.4	0.0	0.5	1.6	2.8	3.1
	Av.	12.7	8.1	12.7	0.5	0.5	1.3	0.8	0.2	4.6	4.1	4.6
Ngochen	1940	5.9	7.2	1.3	5.2	.....	22.1	7.6	4.2	.....	.....	4.9
	1941	3.0	8.9	4.5	5.6	5.2	36.5	18.8	1.9	6.7	6.1	3.6
	1942	8.3	3.6	10.7	3.9	5.5	15.0	21.3	2.5	13.5	12.4	8.2
	1943	7.2	7.3	5.2	1.4	2.9	19.0	5.4	1.9	7.0	9.0	0.7
	Av.	6.1	7.0	5.4	4.1	4.5	23.2	18.3	2.4	9.1	9.2	4.3
Grassland	1940	5.5	7.6	9.2	0.0	.....	0.0	0.0	8.4	.....	.....	17.2
	1941	12.1	10.0	1.3	3.3	1.4	0.0	0.0	16.2	19.9	20.7	23.8
	1942	6.4	12.2	7.9	9.4	0.0	1.6	2.3	23.5	18.4	25.1	15.8
	1943	4.0	9.6	3.5	0.0	4.2	0.0	0.0	9.8	24.4	22.3	26.8
	Av.	7.0	9.9	5.5	3.2	1.3	0.4	0.6	14.5	20.9	22.7	20.8
T. H. No. 3929	1940	41.7	44.9	55.9	33.6	.....	62.1	64.2	37.2	.....	.....	53.7
	1941	26.5	40.1	31.2	48.9	48.1	38.8	77.3	20.3	23.1	44.3	8.6
	1942	55.3	75.2	10.4	21.2	43.6	60.1	86.6	18.2	48.2	40.1	66.4
	1943	22.0	60.0	47.8	40.4	72.4	74.6	78.3	32.7	63.5	59.1	68.4
	Av.	36.4	50.1	36.3	36.0	54.7	61.4	76.6	27.1	44.9	47.8	49.8

[a] Averages of 4 replicated plots.

[b] Number and place of smut collection: 1a, Nanking, Kiangsu; 2a, Wukung, Shensi; 3a, Tinghsien, Hopeh; 4a, Yaotien, Kansu; 5a, Kaifeng, Honan; 1, Kunming, Yunnan; 2 and 3, Loyang, Honan; 4, Kweiyang, Kweichow; 5, Chengtu, Szechwan; 6, Huishiu, Kweichow; 7, Peiping, Hopeh; 8, 9, and 10, Sunghsien, Honan; 11, Malung, Yunnan; 12, Chan-I, Yunnan; 13, Linju, Honan; 14, Tenghsien, Honan; 15, Lushih, Honan; 16, Huahsien, Honan; 17, Tsinan, Shangtung; 18, Suining, Szechwan; 19, Ping-I, Yunnan; 20, Chengkung, Yunnan; and 21 Luiiang, Yunnan. (1a to 5a inclusive were identified in 1936 (3).)

oculate 14 varieties of wheat, including the 4 differential hosts given in table 1, and a variety of poulard wheat known as T. H. No. 559. For comparison, this poulard wheat was also inoculated with the foregoing 11 physiologic races. In the spring of 1943, not a single culm of the poulard wheat, inoculated with these physiologic races, had become smutted. On the other hand, 6.1 per cent of the culms of the poulard wheat, inoculated with the smut from that host, had become smutted. This new smut collection produced different reactions on the common wheats, but the 4 differential hosts, given in table 1, are resistant to it. This additional collection is a distinct race of *Urocystis tritici*. It is, therefore, designated as race 12.

Thus, at the present time, 12 pathogenically distinct races of *Urocystis tritici* have been identified. The varietal reactions that serve to differentiate these races are presented in table 2.

TABLE 2.—*Relative susceptibility*[a] *of 5 differential hosts to 12 physiologic races of Urocystis tritici*

Differential host	Physiologic race No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Common wheat:												
T. H. No. 1932	R	S	R	R	I	R	R	I	R	R	I	R
N. 716	R	R	R	I	I	R	R	R	R	R	R	R
Ngochen	I	R	I	I	I	R	S	I	R	I	R	R
Grassland	R	R	I	I	I	R	R	R	I	S	S	R
Poulard wheat:												
T. H. No. 559	R	R	R	R	R	R	R	R	R	R	R	I

[a] R = 0–5 per cent infection; I = 5.1–20 per cent infection; S = 21.1–100 per cent infection.

For aid in identifying the 12 physiologic races of flag smut, the varieties of wheat used to differentiate them are arranged in the following key:

Key for identification of 12 physiologic races of Urocystis tritici

	Physiologic race
Tsing Hua No. 559 (*T. turgidum*) resistant	
Nanking No. 716 (*T. vulgare*) resistant	
Grassland (*T. vulgare*) resistant	
Tsing Hua No. 1932 (*T. vulgare*) resistant	
Ngochen (*T. vulgare*) resistant	6
Ngochen intermediate	1
Ngochen susceptible	7
Tsing Hua No. 1932 intermediate	8
Tsing Hua No. 1932 susceptible	2
Grassland intermediate	
Ngochen resistant	9
Ngochen intermediate	3
Grassland susceptible	
Tsing Hua No. 1932 resistant	10
Tsing Hua No. 1932 intermediate	11
Nanking No. 716 intermediate	
Tsing Hua No. 1932 resistant	4
Tsing Hua No. 1932 intermediate	5
Tsing Hua No. 559 intermediate	12

DISCUSSION

The amount of smut produced by a wheat plant depends largely on the amount of moisture in the soil and the temperature during the period of germination (1). The time of sowing, therefore, exerts a great influence upon the strength of attack. In a test of physiologic specialization in *Urocystis tritici,* it is important to bear in mind that the optimum conditions for smut infection should prevail. At Kunming, they occur at the optimum time for wheat planting or a little earlier, about October 15 to 20 when the soil temperatures range from 13° to 25° C. Planting in the latter part of November usually results in a low percentage of smut infection. At that time, the low soil moisture content also becomes a limiting factor.

In the previous investigation (4), the identification of races 1, 2, 3, 4, and 5 was based upon the reactions of Nanking No. 716. This variety of wheat was reported resistant to races 1, 2, and 3, but susceptible to races 4 and 5. In the present studies, it is reported intermediate in its reactions to races 4 and 5 and resistant to all the others. In general, the present results confirm those obtained in the previous studies. Since these 5 races of *Urocystis tritici* have been under observation for 11 years, they seem to be stable in range of pathogenicity.

It is well known that environmental factors may affect the response of host varieties to the causal fungus. Rodenhiser and Holton (2), in their bunt experiments at seven experiment stations, have demonstrated that differences in environment may affect the response of some spring wheat varieties to races of *Tilletia tritici* and *T. levis.* In the case of flag smut fungus, the same fact has been observed. For example, H. 1102, a wheat susceptible to races 1 to 5, inclusive, in Nanking (4), was resistant when grown at Kunming. Since the climatic conditions during the growing period of wheat in these two places are so greatly different, it is believed that the host rather than the races show the primary effect of the environment.

As the smut collections used in the present studies were from widely scattered points in China and were so limited in number, it is not yet possible to give the detailed distribution of these races in China. However, 4 of the 7 collections of the smut made in Yunnan have been identified as race 2. Evidently, this race occurs more frequently than the others in this province.

SUMMARY

Twelve physiologic races of *Urocystis tritici* have been separated on the basis of differences in their pathogenicity on 4 varieties of common and 1 variety of poulard wheats. They have been assigned race numbers 1 to 12. Races 1 to 5, previously identified, still remain constant. Races 6 to 12 are described as new.

Races 4 and 5 produce intermediate reactions on Nanking No. 716, a well-known flag smut resistant wheat in China. Race 12, collected on a poulard wheat, is the only race that infects a turgidum wheat.

In Yunnan, 5 races have been identified. Race 2 is conspicuously prevalent and widespread. It occurred 4 times in 7 collections.

Races 1 to 5 have proved stable in pathogenicity for 11 years.

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ELSINOË AND SPHACELOMA DISEASES IN YUNNAN, CHINA, PARTICULARLY HYACINTH BEAN SCAB AND SCAB OF CASTOR BEAN

C. C. Cheo and Anna E. Jenkins[1]

(Accepted for publication January 15, 1945)

Diseases of plants caused by *Elsinoë* (9) and *Sphaceloma* (9) have not been noted heretofore in Yunnan, China; it was expected, however, that investigations would reveal their presence. The five diseases of the group here reported from that Province were found in 1938–1939 by the senior writer or by his colleagues of the Tsing Hua University, following removal from Peiping to Kunming. The first three diseases, previously reported from China and known elsewhere in the world, are rose anthracnose, caused by *S. rosarum* (Pass.) Jenkins (8), grape anthracnose, caused by *E. ampelina* Shear (17), and sour orange scab, caused by *E. fawcetti* Bitanc. and Jenkins (2). Previous records of these diseases in China are assembled; no others have been reported from the country. The other two diseases, hyacinth bean scab and castor bean scab, are essentially new; specimens of both from elsewhere in the Eastern Hemisphere were available to the junior writer when the present cooperative study was undertaken. From its nature the present paper should serve as a background for further work on this group of diseases in China. The precedent of referring to citrus scab in China as sour orange scab is followed, although suitable specimens have not yet been available for comparative determination.

ROSE ANTHRACNOSE

Garden roses at the Cheng-ying Primary School at Shi-ping (Szebing),[2] Yunnan, were severely spotted with rose anthracnose. In China, this disease has previously been reported on wild rose from western Szechwan, north of Yunnan, and from Korea (8).

GRAPE ANTHRACNOSE

In Yunnan viticulture is limited practically to vines planted as a border surrounding the vegetable garden. In August, 1939, leaves and stems of such plants at Pu-Chao, Kai-Yuen, were severely attacked by anthracnose.

In China, outside Yunnan, this disease has been found in widely separated places. Three available records from Nanking consist of a specimen of affected grapes collected in April, 1926, by R. H. Porter (USM 74775),[3]

[1] This paper was presented in greater detail by the senior writer at the celebration on April 18, 1940, at Kunming, Yunnan, of the 30th anniversary of the National Tsing Hua University. Contributions were to have been published in a special number of "Science Reports" of the University commemorating the occasion. Because of war this plan has not materialized.

[2] In this paper place names in parenthesis are those of the Chinese official language (Khan hua) and were furnished by W. T. Swingle.

[3] In this paper the symbol "USM" followed by an accession number refers to specimens in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland.

a literature record of the disease also on berries by Teng (20), and a separate account by Chiao (5). Although not previously reported, in 1937 the senior writer noticed the disease on vines in Shangtung Province. He had earlier reported it as causing serious damage in Hopei Province (4). At Changli in this province "long white grape," the most susceptible variety, showed up to 50 per cent infection. At Peiping the local varieties Rose Fragnant and Confort were attacked, Rose Fragnant so severely so that growth was checked prematurely. Among local varieties differences in susceptibility were apparent.

SOUR ORANGE SCAB

At I-liang, Yunnan, sour orange scab was found on fruit of what was apparently mandarin orange by T. F. Yu, T. H. Wang, and S. T. Chao, who contributed representative specimens. At Nan Chi leaves and young fruit of what was possibly a lemon hybrid also was affected. An isolation of the scab pathogen was made and this is referred to in the section entitled "Parallel cultural comparisons."

Sour orange scab is probably old in China, although early records are not directly available. In Japan, where the disease is believed to have been present "from ancient times," original introduction from China is suggested (*cf.* 19). The earliest available specimen from China consists of diseased rind of "Otehetite orange" from a market in Hong Kong, May 15, 1915, W. T. Swingle (USM 73246). In a recent conversation with Swingle, he stated that this "orange" is probably a hybrid lemon. Mention has already been made (11, Table 1) of scab on the herbarium specimen of *Citrus aurantium*? from New Territory, China, 1928, C. C. Chum 624, in the Arnold Arboretum. Published Chinese records of scab as assembled by Tai (18) are all from South China, *viz.*, Reinking (15, 16) and Tu (21), To these should be added Lu and Cheo's (12)[4] research paper, which reports the first cultural work with *Elsinoë fawcetti* in China. An observation by them not recorded in their paper is given here as follows: Eureka lemon growing in the citrus plantation at Lingnan Agricultural College, Canton, China, where the investigation was conducted, remained unaffected by scab, although growing among such highly susceptible scabbed varieties as Hong-lemon and Pei-lemon. Tu's statement relative to the kinds of citrus affected in South China is: "Scab . . . is coextensive with the growing of the Chinese lemon (*Citrus limonia* Osbeck). The young leaves, twigs and fruits are usually attacked. All the varieties are equally susceptible. All the citrange varieties . . . are extremely susceptible when young. Both Sweet orange and Mandarin orange are either only slightly affected or remain highly resistant under field conditions."

While in Indo-China in 1939, the senior writer noticed scab on Mandarin orange (?) growing in orchards at Lao-kai, Province of Tungkin. The disease appears not to have been reported heretofore from Indo-China, although

[4] The junior writer is indebted to Dr. Swingle for an English translation of this article from the original Chinese, made in March, 1939, by E. J. Hagerty, University of California.

two specimens are available. The first consists of leaves of Mandarin orange (?) collected on May 19, 1920, by Reinking (USM 69196), the other of citrus rind preserved by R. Kent Beattie from fruit served him at table during his stay at Tourane, Province of Annam, in 1930 (USM 73247).

SCAB OF HYACINTH BEAN

In August, 1938, more than 15 per cent of pods of hyacinth bean (*Dolichos lablab* L.) in a market in Tali, western Yunnan, were affected by the disease here termed "scab of hyacinth bean." Visiting the fields at Er-kai (Erh-hai) where the crop was grown, it was found that all aerial parts of the plant were attacked, severely affected young pods and tendrils having failed to develop or having been killed outright. This same disease was observed also in market in Kunming, both in 1938 and 1939.

It seems probable that this scab of hyacinth bean in Yunnan is the same as that discovered in Kenya Colony (1930) and in Uganda, Africa, a decade or so ago. The finding in Kenya was by the local mycologist E. J. Edwards, who sent a specimen to E. J. Butler, then Director of the Imperial Mycological Institute. Upon finding asci entirely typical of *Elsinoë* on this material Dr. Butler communicated with the junior writer, to whom part of the specimen was later sent by Dr. S. F. Ashby (USM 72652) in 1936. Hansford's (6) reports of the disease in Uganda are here quoted for convenience of reference as follows:

"The cover crop of *Dolichos lablab* suffered severely from attack by a fungus at present unidentified, but probably related to *Sphaceloma citri*[5] causing the scab disease of citrus. Many of the plants were killed off completely and most showed severe damage to foliage and aerial parts. Affected plants produced very little seed."

"The disease of *Dolichos lablab* reported in 1932 as due to *Sphaceloma sp.*, again occured at Serere. Plots of *Canavalia ensiformis* also showed infection. Other legumes were not affected."

A specimen of the *Elsinoë* on *Canavalia* from Serere was sent to Dr. Butler, and later divided with the junior writer (USM 72563). The label gives the host as *C. ensiformis* (?). Dr. Butler's identification of this specimen as *E. canavaliae* Rac. (14), which is known only on *C. gladiata* (Jacq.) DC., *i.e.*, sword or saber bean, is probably correct. In view of the fact that the sword bean and jack bean (*C. ensiformis* (L.) DC.) have been greatly confused as shown by Piper (13, also see 7, p. 1), it seems probable that the diseased *Canavalia* at Serere was sword bean rather than jack bean. Cross inoculations on both these legumes with the pathogen of hyacinth bean scab made at Yunnan will be reported later. In Yunnan the senior writer has not found *Canavalia* planted in fields with hyacinth bean; however, in one instance at the Tropical Agricultural Experiment Station at Ho-kow (Ho-K'ou) he observed sword bean growing as a shade plant over hyacinth bean seedlings. The identification of the *Canavalia* as sword bean was based on

[5] The fungus referred to here is *Elsinoë fawcetti*.

Piper's classification (13), *i.e.*, pods about four times as long as broad, 20–25 cm. long, with white seeds.

Symptoms

As observed in Yunnan, the leaf spot is more conspicuous on the lower leaf surface; on the Edwards specimen from Kenya, however, the situation is reversed. On the dry specimens in both instances spots are often "light

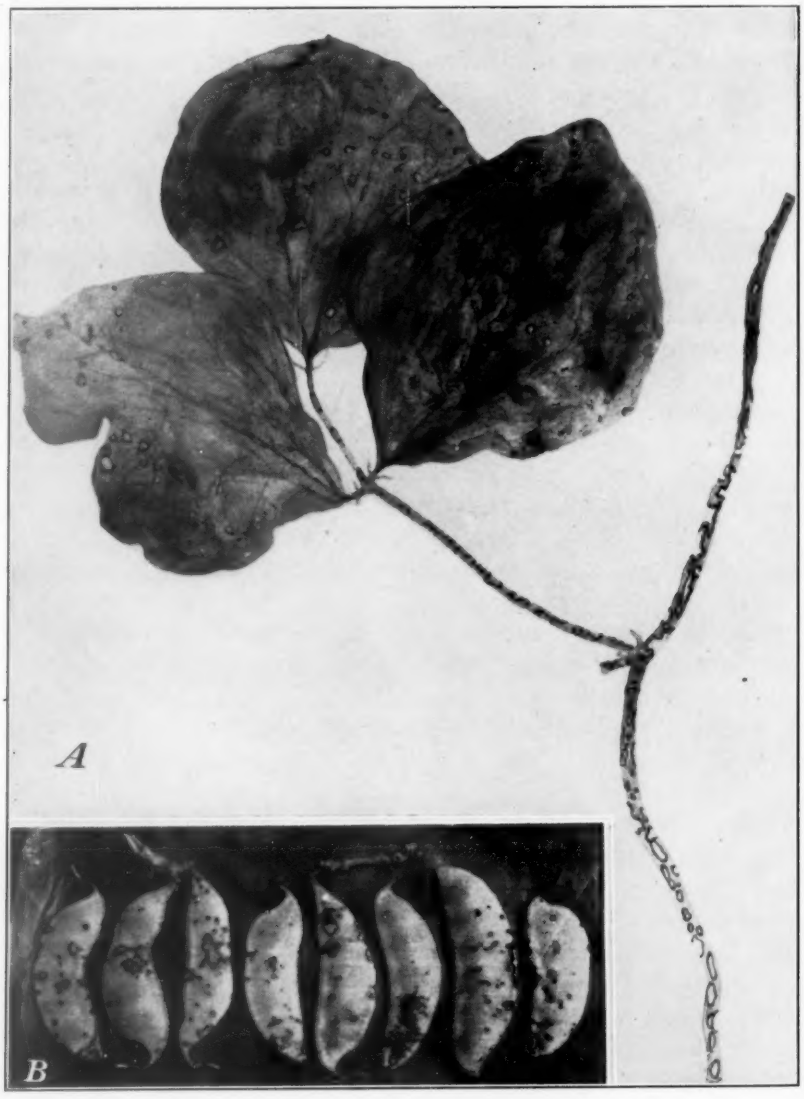

FIG. 1. Scab on lower surface (A) and on pods (B) of hyacinth bean from Yunnan. A, approximately natural size; B, reduced. Photographs by Cheo (A) and C. N. Cheng (B).

buff,"[6] sometimes faintly bordered with "burnt sienna," and they are often raised at the margin. In their distribution over the blade surface they tend to follow the venation, often involving also the adjacent nonvascular tissue near the midrib or lesser veins. The midrib from the base of the blade practically to the apex may be affected, and the entire apical area also attacked. Where several veins in the same part of the leaf are involved, there may be a general yellowing of the leaf in that region. Interveinal lesions occurring separately tend to be circular, although at the junction of veins, they may be irregularly star-shaped. They may reach 4 mm. in diameter. Symptoms on petioles are similar to those on stems (Fig. 1, A).

On the stems the cankers are circular to linear. They may be numerous and scattered, grouped, or coalescent. Individual lesions range from minute spots to cankers 3 mm. wide by 1 cm. long. They may be flat or slightly depressed, but are often raised, at least at the margin. They are brown at first, becoming ashen, narrowly bordered with pale yellow, or sometimes with dark purplish black (Figs. 1, A, and 2, A).

Pod lesions are more or less circular, punctate to about 5 mm. in diameter. They may be scattered, grouped, or coalescent and where abundant they may produce a mottling effect over practically the entire surface of one or both valves. The suture also may be attacked and lesions here be joined linearly. The spots may be somewhat raised, although not necessarily so. They are brown to purplish brown, becoming lighter at the center, and may be bordered by a band of dark purplish black. The pedicel and calyx as well as the valves may be affected (Figs. 1, B, and 2, A).

The pathogen of hyacinth bean scab (Fig. 2, D, E, F, and Fig. 3) from the sources indicated is here treated as a single species and is described as follows:

Elsinoë dolichi Jenkins, and Bitancourt and Cheo.[7] Ascomata amphigenous, but more abundant on the leaf surface originally infected, on opposite surface produced on or near veins, viewed superficially, appearing as elevations in the dark stromatic rind covering the lesions, also occurring more or less separately as dark punctate bodies, visible as light circular areas where they have caused a rupturing of the rind; in cross section constituting small hyaline to pale yellow pseudoparenchymatic masses often pulvinate and erumpent, with the asci sometimes in one layer although not necessarily so limited, 60 to 300 μ in diameter by as many as 100 μ high, often coalescent or at least adjacent; asci subglobose, pyriform to ellipsoid, 20 to 32 μ in vertical diameter by 15 to 22 μ broad, ascospores (immature) 1-septate, probably becoming 3-septate upon maturity, hyaline; imperfect stage consisting of a more or less continuous dark stroma disrupting the epidermis, and developing into the more or less well developed dark stromatic rind, spreading more or less deeply

[6] Color readings in quotations, are based on R. Ridgway, Color Standards and Color Nomenclature, 43 p., Washington, D. C., 1912.

[7] Because of the unavoidable delay in publishing this article the diagnoses of the two new species in Latin were published separately (10).

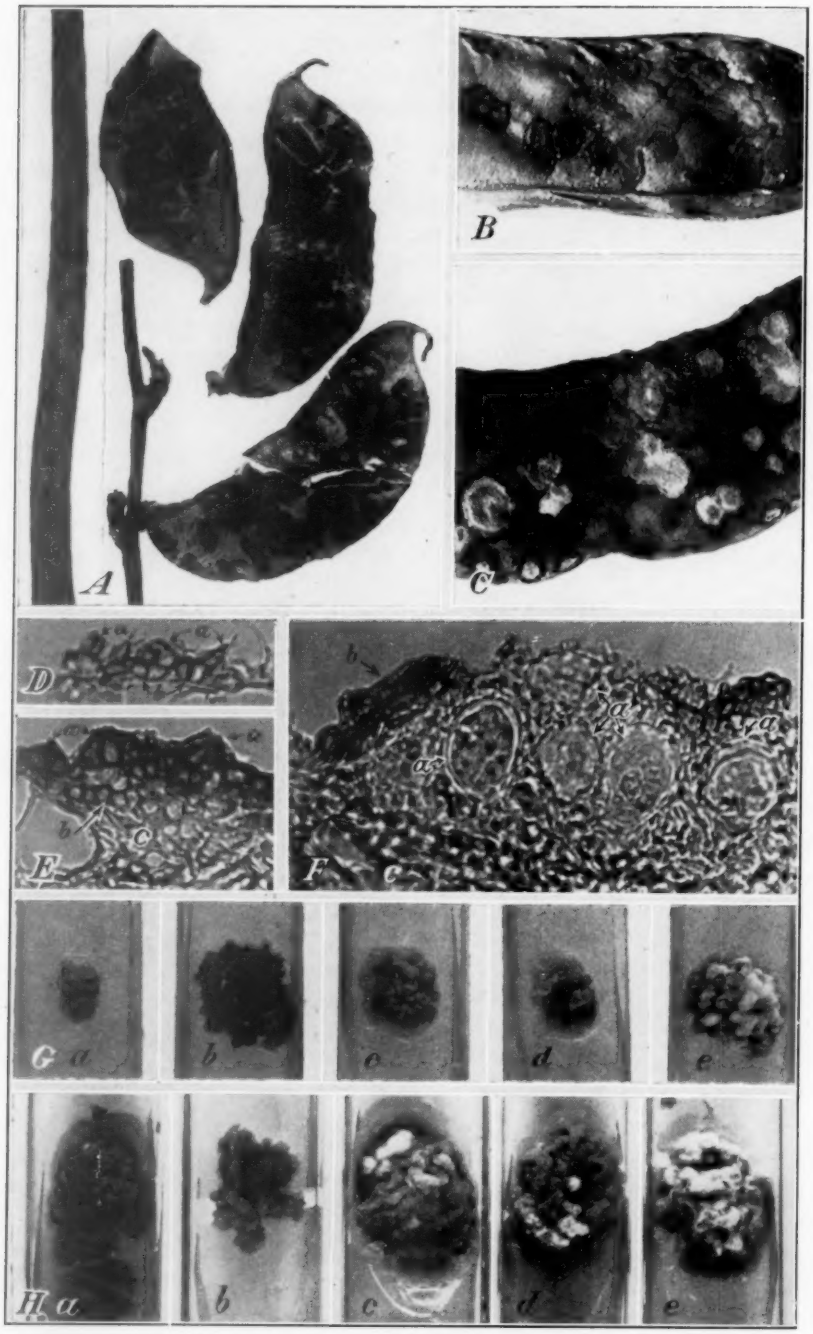

FIG. 2. Scab on pods and stems of hyacinth bean, Kenya Colony, Africa, Sept. 17, 1930. D. C. Edwards. Comm. S. F. Ashby in 1936. B and C. Pod lesions of related legume diseases: (B) scab of *Canavalia* (supposedly sword bean), Los Banos, P. I., Mar. 7, 1920. G. O. Oefemia (USM 69188); (C) lima bean scab, Palo Seco, P. R., Apr. 12,

into the tissue of the lesions as a hyaline to yellowish prosenchyma, superficially often covered with a well defined dark layer of conidiophores, although for short distances it may be pale, rind including conidiophores attaining 30 μ in thickness, or even in absence of well developed conidiophores sometimes nearly 50 μ thick, composed of small often rounded cells; conidiophores, sometimes arranged in small closely grouped fascicles, consist individually of indefinite to more or less abruptly tapering structures up to 20 μ high and 3.6 to 5.3 μ broad at the base; conidia seen in small numbers only, minute, up to 3.5 μ in diam.; as observed in culture (by Cheo), spherical (2.5–3 μ in diam.) to elliptical (3–7.6 by 1.5–3 μ), hyaline.

Distribution: On leaves, stems, and pods of hyacinth bean (*Dolichos lablab* L.) causing the disease termed "scab of hyacinth bean," Kenya and Uganda, Africa, and Yunnan, China.

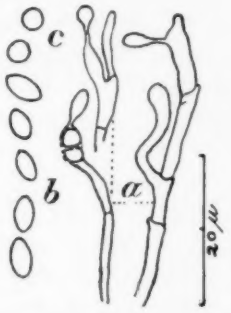

FIG. 3. *Elsinoë dolichi*. *a*. Conidial formation from hyphae in a six-day-old potato-agar culture, *b* and *c*, conidia. Drawing by Cheo.

Specimens examined: Kenya Colony, Sept. 17, 1930, D. C. Edwards, Type (USM 72652), Phytopathological Herbarium, Instituto Biologico, São Paulo, Brazil (3267), and Imperial Mycological Institute, Kew, Surrey, England; Yunnan, China, Nov. 10, 1938, C. C. Cheo (USM 73032).

Cultures

Elsinoë dolichi was isolated in Yunnan and there grown on potato, malt, and corn-meal agar. The growth was wrinkled and variously colored. On corn meal the main or central part of the culture was covered with livid pink aerial hyphal growth; the agar became violet. The subculture sent to the junior writer was compared with cultures of other species from legumes, namely *E. phaseoli* Jenkins (3) on lima bean (*Phaseolus lunatus macrocarpus* Benth.) from Cuba and *Sphaceloma arachidis* Bitanc. & Jenkins (1)

1939, W. A. McCubbin, Comm. W. A. M. in 1939 (USM 73070). Both A and B, ×1. D–F. *Elsinoë dolichi* on upper surface of a leaf spot on the Edwards' specimen of hyacinth bean. D. Conidiophores arising from a single hyphal strand (*a*) or layer (*b*). E, *a*, Three clumps of conidiophores; *b* and *c*, stroma developed within the disorganized host tissue. F. Ascomycetous stage showing asci (*a*) imbedded in hyaline stromatic tissue and (*b*) ruptured epithelium corresponding to the conidial layer, *c*, host tissue. G. Young cultures, and H, older cultures on potato-dextrose agar; *a*, *E. dolichi*; *b*, *E. phaseoli*; *c*, *Sphaceloma arachidis*; *d*, *S. ricini*; *e*, *E. fawcetti*. All ×1. Photographs (A–H) by M. L. F. Foubert.

on peanut (*Arachis hypogea* L.) from São Paulo, Brazil. The culture from lima bean was isolated by the junior writer and that from peanut by Bitancourt as shown in the section entitled "Parallel cultural comparisons." The three cultures were distinct.

Inoculations and Cross-inoculations

Hyacinth bean was inoculated with *Elsinoë dolichi* at Kunming in June and July, 1939. Cultural growth on potato agar was used as inoculum. This was macerated and placed on wet pads of cotton, which were applied to the surface to be inoculated, with the inoculum in contact with the plant. The cotton was kept wet for 48 hours by sprinkling it frequently. At the end of this period it was removed together with the inoculum adhering to it. In the first experiment 15 tender stems, 20 young leaves, lower surface, and 18 young pods were inoculated. In each instance over 50 per cent infection was obtained. Typical scab lesions were distinct on leaves in 9 days and on pods and stems in 12 days. In the second experiment, in which the upper surface of 16 young leaves was inoculated, the results were completely negative. The controls, 15 in the first experiment, 14 in the second, all remained healthy.

In cross-inoculation experiments in Cuba with *Elsinoë phaseoli* this lima-bean pathogen failed to infect hyacinth bean, sword bean, jack bean, and several other legumes (3). Sword bean and jack bean are uncommon in Yunnan; seed of sword bean were available from the plants at Ho-kow, and those of jack bean and lima bean were obtained elsewhere. Plants grown from these seed were inoculated with the culture of *E. dolichi*. The method was the same as that employed for inoculating hyacinth bean, except that both leaf surfaces were inoculated. The results were entirely negative as in the case of the cross-inoculation experiments in Cuba with *E. phaseoli*. Figure 2, B, represents natural pod infection of *Canavalia*, presumably sword bean, by *E. canavaliae;* figure 2, C, that of lima bean by *E. phaseoli*. The specimens are cited in the legend.

SCAB OF CASTOR BEAN

Castor bean (*Ricinus communis* L.) has long been cultivated for seed in widely scattered localities in southern Yunnan, where it grows as a perennial. Varieties are classified according to petiole color and capsule characteristics.

In June, 1939, the senior writer observed "scab of castor bean" at Gee-kai (Chi-kai) Station and later, in August, saw plants severely attacked at Ta-chong (Ta-Chaung). He had already noticed lesions of the scab on herbarium specimens of castor bean collected at Ta-chong on December 18, 1938, by Yu, Wang, and Chao. In these cases three varieties were affected; Red petiole with small thorny capsule, Red petiole with large thorny capsule, and Green petiole with large thorny capsule. Other varieties that may be affected, as well as actual range of the disease in Yunnan remain to be determined.

Before the disease was discovered in Yunnan it had been found in Formosa by K. Sawada, who sent specimens to the junior writer. These also were severely diseased.

Symptoms

Spots are more prominent on the upper leaf surface and are generally, although not always, visible below. They may be scattered over the blade including the margin; often, however, they are concentrated along the main and lesser veins. At first they appear as small water-soaked areas, which soon turn reddish brown, finally becoming pale, "tilleul buff" and "vinaceous buff," bordered by brown. Where not influenced by veins, individual spots are more or less circular, 2 to 3 mm. in diam. Near veins they may be elliptical to elongate or triangular and on the lower leaf surface in particular, irregularly star-shaped. Vein lesions, below, are inconspicuous, elliptical to linear depressed areas 1.5 mm. wide by 1 cm., more or less, in length. Severe attack along a rib may prevent its normal elongation, with the result that the blade tissue on either side, continuing its growth, becomes wrinkled or waved, and sometimes severed from the diseased rib tissue. The dead papery spots may fall away and the leaf become variously lacerated and torn, as well as blighted where severely attacked (Fig. 4, A–C).

Spots on the petiole and stem are elliptical to elongate, sometimes pointed at each end, reaching 8 mm. in width by 1.5 mm. or more in length. They are at first reddish brown but later become pale, "tilleul buff," at the center, with a brown or purplish black border. This light colored central part may become broken and crumble away. The spots are even with the surface of the petiole or stem, or they may be slightly depressed. They may occur singly, or be grouped and more or less coalescent (Fig. 4, D).

The Pathogen

On the basis of the present study the pathogen of castor bean scab (Fig. 5) is described as follows:

Sphaceloma ricini Jenkins and Cheo.[8] On cankers, conidiophore palisade, yellowish to amber, sometimes practically continuous as a pale dusky layer over all the bordering area, occasionally arched away from the hyperplastic tissue of the canker, with slight or not appreciable pseudoparenchymatous base, 10 to 30 μ, often 25 to 30 μ in thickness, individual clumps of conidiophores sometimes discernible, and where the conidiophores are closely appressed the more slender apices often remaining free from contact with one another, individual conidiophores also sometimes standing apart from one another, observed on leaf spots as well as on cankers, awl-shaped to cylindrical, unbranched, or rarely forked, occasionally abruptly bent, smooth, or apiculate from acropleurogenous production of conidia, apically more or less abruptly pointed, blunt or smooth, where conidia have been produced sometimes spear-shaped, hyaline to pale yellow;

[8] Because of unavoidable delay in publishing this article the diagnoses of the two new species in Latin were published separately (10).

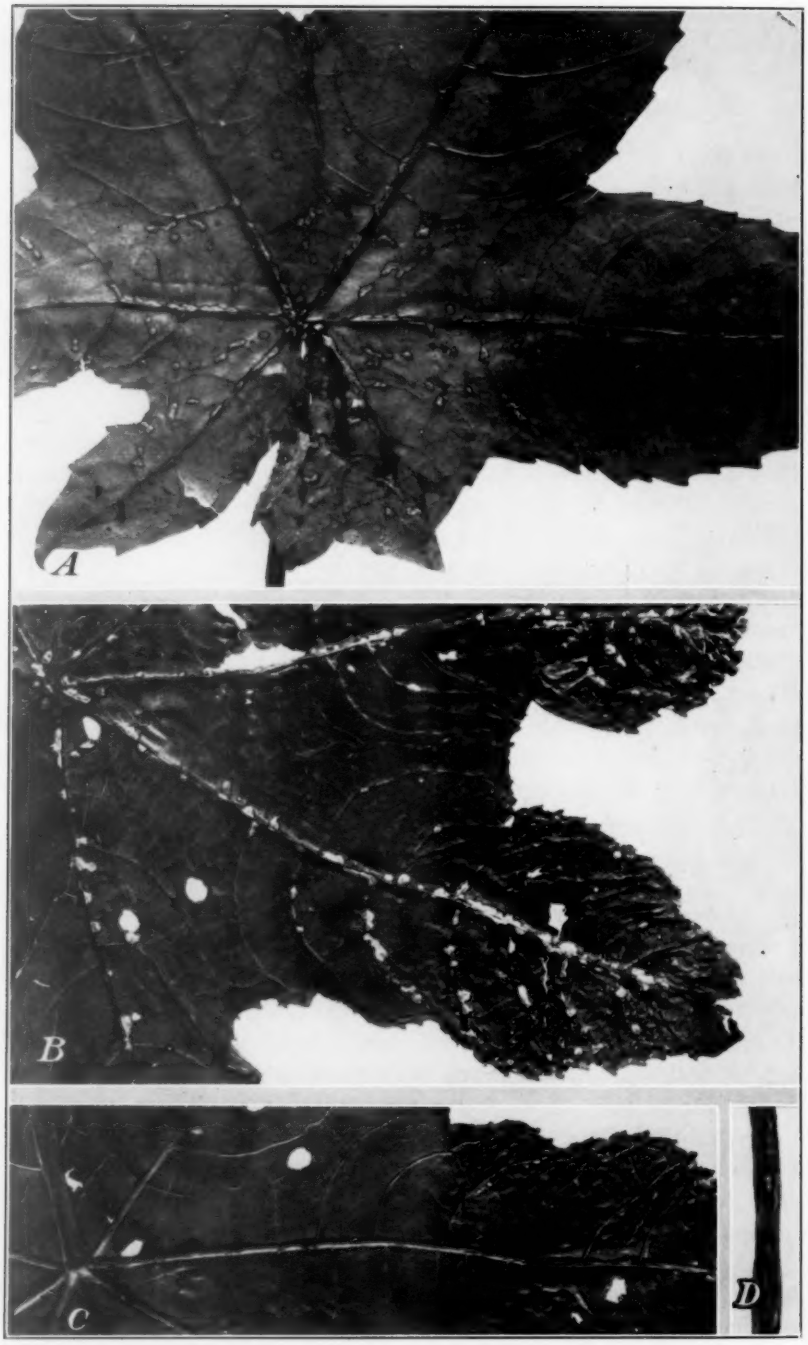

FIG. 4. Leaf spots of castor bean scab. A and B. Upper leaf surface; C. lower surface of part of B. D. Petiole lesions. Specimen from Ta-Chung, Yunnan, C. C. Cheo, Aug. 2, 1939. ×1. Photograph by Foubert.

conidia of various shapes and sizes, oblong to ovoid, elliptical, or fusiform, minute (1–2 μ), up to 10–15 by 2.6–4.5 μ, smaller conidia hyaline, larger and fusiform conidia yellowish.

Distribution: On leaves and stems of castor bean (*Ricinus communis* L.) causing the disease termed "scab of castor bean," Taihoku, Formosa (Taiwan), and several localities in Yunnan, China.

Specimens examined: Taihoku, Formosa, July 2, 1938 (Type, USM 72921) and August 23, 1938 (USM 72934); Ta-chong, Yunnan, China,

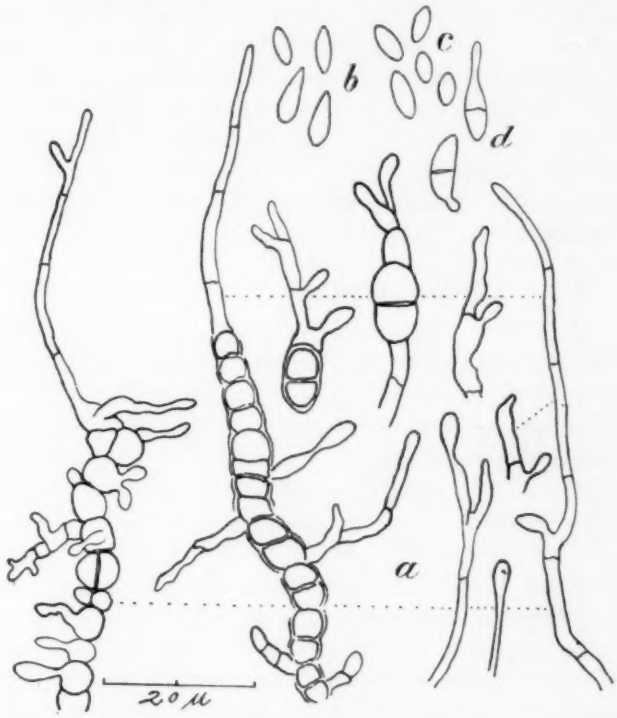

FIG. 5. *Sphaceloma ricini*. *a*. Conidial formation from hyphae in six-day-old potato agar culture, *b*, *c*, *d*, conidia. Drawing by Cheo.

December 16, 1938, T. F. Yu, T. H. Wang, and S. T. Chao (Herb. Nat. Tsing Hua Univ.), Aug. 2, 1939, C. C. Cheo (USM 73162); Gee-kai, Yunnan, June, 1939, C. C. Cheo (Herb. Nat. Tsing Hua Univ.).

Cultures

Sphaceloma ricini was isolated at Kunming and grown on potato, malt, and corn-meal agar. A black sector produced in one instance was isolated and remained constant throughout many transfers. A black sector also developed from the subculture sent the junior writer. The aerial hyphae of the culture on corn meal were white, and the medium became violet, although not the same shade as in the case of the corresponding culture of *Elsinoë dolichi*. Conidia produced in culture at Yunnan are represented in figure 5.

Inoculations

The method of inoculating castor bean plants was essentially the same as that employed in the experiments with *Elsinoë dolichi*. In general the upper leaf surface was inoculated, as this is obviously more susceptible to infection than the lower leaf surface.

Infection was obtained on the four varieties inoculated, namely, Green petiole variety with large smooth capsules and that with large thorny capsules, and the Red petiole variety with large thorny capsules and that with small thorny capsules. Good infection was obtained in all cases except in the instances where the inoculum was placed on the lower leaf surface or where the inoculum was the black strain of the fungus. The black strain, originating as a saltation, was a weak pathogen. The time required for the lesions to become visible or distinct ranged from 7 to 15 days with one instance of leaf discoloration in 2 days. The lesions in all cases were entirely typical of those of scab on castor bean observed in nature. Controls for each of the experiments remained healthy.

PARALLEL CULTURAL COMPARISONS

The parallel cultural comparison is represented in figure 2, G, *a* to *e* and H, *a* to *e*. The first series of these Thaxter's potato agar cultures[9] grew for two weeks at room temperature. In the second series, which is a separate set of transfers, all but H, *c*, were grown one month at room temperature, then held for two months at a lower temperature (refrigerator) which permitted slight growth. These cultures were thus three months old when photographed. The culture in H, *c*, represents six weeks' growth at room temperature.

Elsinoë dolichi, *E. phaseoli*, and *Sphaceloma arachidis*, in the order named, are represented in figure 2, G, *a–c*, and H, *a–c*. The two-week-old cultures (Fig. 2, G, *a–c*) were of the following colorations: *E. dolichi*, "clay color," *E. phaseoli*, "carbon brown," *S. arachidis*, reading from the outside to the center, "cinnamon buff," "avellaneous" and "buffy brown." Throughout their development the cultures in figure 2, H, *a–c*, remained distinct from each other; that of *E. dolichi* became chromogenic, "sorghum brown." At the age of 8 months the thallus of *E. dolichi* had become "Victoria lake" to nearly black; that of *E. phaseoli* was "fawn color" at the center and in other parts, "Hessian brown," "diamond brown," and black. Comparable growth of the corresponding culture of *S. arachidis* was generally drab, *i.e.*, "vinaceous drab," "light Quaker drab," and "Quaker drab."

[9] Thaxter's potato agar was made by Miss Agnes Quirk of the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, as follows: 500 g. potato (mature and sound), 1,000 cc. distilled water, 20 g. dextrose, 15 g. agar. Slice potatoes thin, immediately add distilled water, steam 20 min. in the Arnold sterilizer, or let simmer in a water bath, temperature below 60° C., for one hour. Filter through cheesecloth, make up to original amount with water. Add 2 per cent dextrose and 1½ per cent agar and cook for one hour; filter through cotton, tube, and autoclave tubes 20 min. at 115° C.

Figure 2, G, *d*, and H, *d*, represents the culture of *Sphaceloma ricini*. At the end of the first month the culture in H was "ochraceous brown" in the outer part and "Mars brown" at the center, with the medium chromogenic, "lime green" and "mignonette green"; after 8 months, thallus "cinnamon" with a mixture of other colors, medium uniformly "mignonette green."

The culture of *Elsinoë fawcetti* in the two series (Fig. 2, G, *e*, and H, *e*) was of essentially the same coloration; at the end of one month "ochraceous buff" at the outside and "walnut brown" deepening to "maroon" at the center; aged 8 months, "ochraceous tawny" in the outer part, "pale vinaceous drab" and "light vinaceous drab" over a large area at the center, where there were aerial hyphae.

SUMMARY

Five diseases caused by *Elsinoë* and *Sphaceloma* were discovered by the senior writer in Yunnan Province in 1938–1939. These are rose anthracnose, grape anthracnose, and sour orange scab, of which previous records elsewhere in China are assembled. The two other diseases, hyacinth bean scab and scab of castor bean, are essentially new. Without description, the presence of hyacinth bean scab in Uganda, Africa, had been reported by Hansford (1932–33); also part of the D. C. Edwards' specimen from Kenya Colony, 1930, had been made available to the junior writer (1936). Similarly, Sawada had sent specimens of castor bean scab from Formosa prior to the receipt of Cheo's specimens from Yunnan. Symptoms of these two new diseases are delineated and their pathogens described, the organism from hyacinth bean as *Elsinoë dolichi* Jenkins, Bitanc. and Cheo, that from castor bean as *Sphaceloma ricini* Jenkins and Cheo. (Diagnoses in Latin were made available in 1941.) Inoculations with cultures of *S. ricini* on four different castor bean varieties gave positive results, as did corresponding experiments on hyacinth bean with *E. dolichi*. Only negative results were obtained in cross inoculations with *E. dolichi* on sword bean, jack bean, and lima bean. Parallel cultural comparisons on Thaxter's potato agar included one culture each of *E. dolichi*, two other legume species, *E. phaseoli* on lima bean from Cuba and *S. arachidis* on peanut from São Paulo, Brazil, *Sphaceloma ricini* and *E. Lancetti*. The cultures were distinct in all instances.

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THE FIELD INOCULATION OF RYE WITH CLAVICEPS PURPUREA[1]

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INTRODUCTION

This paper presents a new technique for the inoculation of rye with *Claviceps purpurea* (Fr.) Tul. The method consists primarily of producing an artificial honeydew by mixing saprophytically grown spores with a strong sugar solution. In this solution the spores can be stored for months without great loss of viability. Spores do not die when drops of the sugar-spore-suspension dry out, and will germinate readily when water is added. In these respects the suspension resembles natural honeydew. A special sprayer was devised to apply the diluted spore suspension to the blooming rye plants. An abstract of this paper was previously presented to Phytopathology (9).

The results of this work may be applied to commercial production of ergot and to many scientific problems such as the relation of insects to ergot, the testing of grasses bred for resistance to ergot, the taxonomy of species of *Claviceps*, and the study of physiologic specialization within a species. An artificial matrix suspension might be used for other fungus or bacterial plant pathogens whose reproductive cells are naturally produced in a matrix.

REVIEW OF THE LITERATURE DEALING WITH THE LARGE-SCALE INOCULATION OF RYE WITH ERGOT

Fairly successful methods for the large-scale inoculation of rye with ergot have been worked out by Bekesy (3) and Hecke (5, 6). Bekesy's method is based on the use of a horse-drawn, multiple injecting apparatus which deposits spores inside the closed flowers, while Hecke's method depends upon a considerable amount of hand manipulation to cause the flowers to open and to apply the spores. Hecke's method could not be used for commercial production of ergot in the United States because it requires too much hand labor. Bekesy's apparatus might be adapted to commercial production provided the machine does not cause too much injury to the rye, provided it will stand the wear and tear of much use, and provided most of the infections arise from the initial inoculations. Any method depending upon natural spread is not reliable enough for the propagation of ergot in most locations where rye is grown.

McCrea (10), Hynes (8), and Thomas and Ramakrishnan (11) have tried large-scale inoculations by spraying aqueous spore suspensions on the rye when the plants were in bloom. McCrea's results using a horse drawn

[1] Condensation of a thesis presented to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the Degree of Doctor of Philosophy. Approved by the Director of the Michigan Agricultural Experiment Station as Journal Article No. 721 (n.s.).

sprayer were so inconclusive that she says in her summary, "Field demonstrations have shown it to be improbable that parasitic culture of *C. purpurea* on a large scale would be desirable, under prevailing conditions in Southern Michigan." Hynes says of the results secured by application of an aqueous spore suspension by various means on 200 acres of rye: "Dry seasonal conditions generally were adverse to ergot formation. Consequently, yields were low (½ lb. to 20 lb. per acre), but ergots did develop in most instances—even in the driest areas sown to rye." Thomas and Ramakrishnan sprayed their plots twice and a few infections occurred after the first treatment. Ergot subsequently spread to the whole field, even to the untreated plots. Their results were better than those of McCrea and Hynes, but most of their sclerotia apparently arose from secondary infections, hence a good yield would be dependent upon favorable weather.

Many investigators have had success in small plots by spraying on an aqueous suspension of spores. The results of Fron (4) and Hynes (8) illustrate this. Heckt (7) was granted German and U. S. patents on a hand-operated, multiple injecting apparatus for inoculating rye through the palets and lemmas of closed flowers. He claims that large-scale inoculations can be made, but here, again, much hand labor would be necessary. Barger (2) and Bekesy (3) give good reviews of the literature dealing with attempts at large-scale inoculations with ergot. They also discuss an important phase of the problem not touched upon in this work: the use of more susceptible varieties of rye in order to get maximum infection with minimum inoculation effort.

SEARCH FOR A SPORE MATRIX AND STORAGE OF SPORE SUSPENSIONS

Observations in the field and information gained from the literature gave rise to the hypothesis that the most effective agent for inoculating rye with *Claviceps purpurea* would be a spore suspension with properties similar to the sphacelial suspension produced in nature. To be similar, an artificial suspension must: (1) prevent immediate germination, (2) protect the spores from death by desiccation after application, (3) attract insects, (4) allow germination once the spores come in contact with the pistils of the rye flowers.

Maple sirup, light and dark corn sirups, honey, and a concentrated solution of beet sugar were tested to determine their effect on the vitality of spores suspended in them. Only the beet sugar gave promising results. Table 1 gives the percentage germination of samples removed from quart bottles of 50 per cent beet-sugar-spore-suspension. Spores in a sugar solution matrix will remain viable for months at a low temperature. In tests where germination decreased considerably, large numbers of bacteria were usually present and the decrease was attributed to them.

The percentage germination was determined at first by estimation and later by a smear method. A drop of suspension was placed on a grease-free, sterile slide, smeared to form a thin film, and the slide placed on wet paper in a Petri dish. After incubation for about 18 hours at 28° C., the slide was

TABLE 1.—*Percentage germination of samples from sugar-spore-suspensions stored at various temperatures*

Days suspension in storage before samples taken	Storage temperatures, degrees C.				
	−18	0	12–16	20–26	30
3	60	80	60	70	60
18	40	60	50	0	0
38[a]	70	70	0	.....	.....
42	40	40	.....	.....	.....
61	40	43	.....	.....	.....
128	45	19	.....	.....	.....

[a] Percentages estimated on samples up to 42nd day; others determined by actual count.

allowed to dry. A cover slip with a small drop of water was placed on the slide. Ten high-power fields were counted, combined, and the percentage of germination computed. From time to time the sugar-spore-suspensions in storage were examined and no germination occurred, not even in those stored at room temperature.

In sugar concentrations from 34 to 66 per cent, spores remained viable even when drops of the suspensions were allowed to dry in room air or over calcium chloride for 5 days. Neither the variation in sugar concentration nor the drying of the suspension reduced the percentage germination. This is important because it means that spores applied in the field will remain viable for a number of days even though the weather is dry.

PREPARATION OF SPORE SUSPENSION

Cultures for the mass production of conidia were grown in quart milk bottles using the method of Hynes (8): 250 ml. of wheat and 250 ml. of water were mixed in a bottle, allowed to stand overnight, and autoclaved one hour at 15 lbs. A piece of a heavily sporulating stock culture was added and mixed thoroughly with the wheat medium by pounding on a rubber stopper. All cultures were grown at room temperature and harvested at the end of five to six weeks. The cultures were mixed with an equal volume of tap water and beaten in a blender, a method of preparing inoculum described by Andrus (1). After blending for about two minutes the suspension was screened through a 16-mesh and then a 40-mesh screen. To this thick, water suspension of spores, medium, and mycelium an equal weight of beet sugar was added and stirred until dissolved. Five gallon honey cans were used for storage at −18° C. and 0° C. About 4½ gal. of the sugar-spore-suspension was made from 10 quarts of culture.

FIELD INOCULATIONS

A field trial in 1941 using traps for catching flies, exposing them to a culture of *Claviceps purpurea* and releasing them, failed completely to bring about any infections, but did give rise to the basic hypothesis of this work. The first trials using a sugar-spore-suspension for inoculation were made in

1942 and on four plots an average of 9 per cent of the heads were infected as compared to 1 per cent on the control plots. In 1943, five fields were inoculated, three with a power sprayer and two with a hand sprayer. Two of these fields are described here, with only a few statements about the others.

The Clark Field

This field of Rosen rye contained six blocks in a row. Each block was 80 ft. long and made up of five lengthwise plots 6 ft. wide arranged with roadways to make them accessible to a sprayer. The spraying was done with a small orchard sprayer (Fig. 1). The boom carried besides the nozzles three

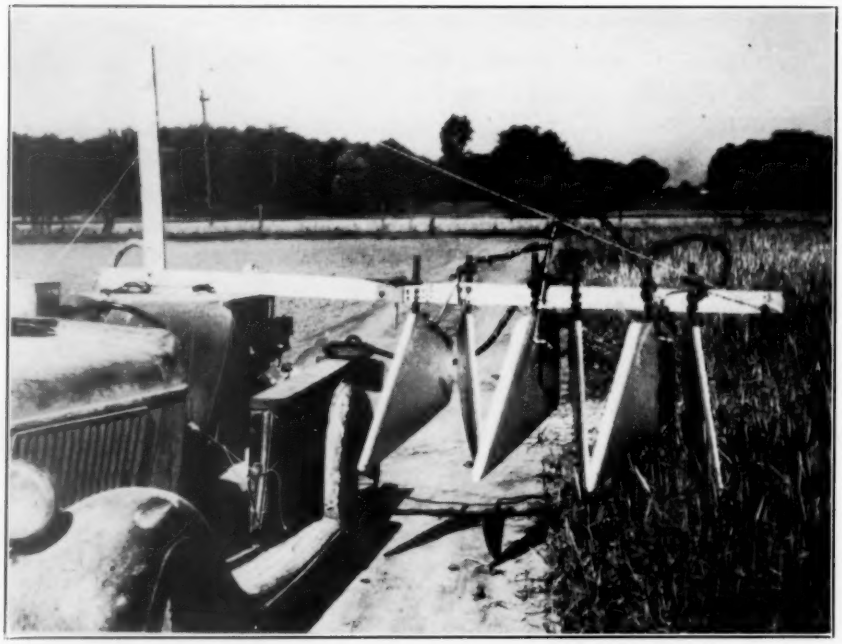

FIG. 1. The power sprayer used to apply the sugar-spore-suspension to the rye flowers.

pairs of guides whose construction and arrangement can be seen in the picture. At the time this field was sprayed there was only one nozzle between each pair of guides and it was adjusted to point into the tops of the rye heads as they passed between the guides.

Information concerning the number of treatments and strength and quality of spore suspension is in table 2. The plots were sprayed once each day on June 8, 9, and 10. Plots in blocks I and V were sprayed once more on June 11. Spraying was done in the morning except on June 9 when the field was treated in the early afternoon. Duplicate plots in blocks I, II, and IV were combined and in blocks V and VI all plots were treated alike. The middle plot in each block was untreated.

In every block, regardless of the kind of treatment, there was a great

difference between the sprayed and unsprayed plots. The lower quality suspension, with 10 to 30 per cent germination, was less efficient in producing infections than was the better quality one. The highest dilution of suspension used was just as effective as the more concentrated suspensions. This substantiates similar results from another experiment. The control plot in all treated blocks was in immediate contact with one treated plot and separated from the next by a 6-ft. roadway. Carry-over was small, but probably accounts for a part of the infection in the controls. The higher percentage of ergot in block III as compared to the controls in the treated blocks was probably due to its location in a lower part of the field. With the commer-

TABLE 2.—*Treatments and results on Clark Field*

Block No.	Number of treatments[a]	Plot	Strength of suspension	Percentage of heads infected[b]
I	4	A	1 : 1	43
		B	1 : 3	38
		C	(untreated)	5
II	3	A	1 : 1	36
		B	1 : 3	30
		C	(untreated)	3
III	0			8
IV	3	A	1 : 7	33
		B	1 : 15	35
		C	(untreated)	3
V	4	A & B	1 : 1	25
		C	(untreated)	6
VI	3	A & B	1 : 1	22
		C	(untreated)	6

[a] Blocks I, II, and IV were treated with spore suspensions whose germination was 40–60 per cent; blocks V and VI with suspensions of 10–30 per cent.
[b] An average for the whole field of 1.8 sclerotia per infected head.

cial production of ergot in mind, it is noteworthy that the treated area was more than one quarter of an acre and that a rapidly moving sprayer applying a sugar-spore-suspension inoculum caused a fairly high percentage of infection. This was the first attempt to apply this type of suspension with a power sprayer and the whole operation was crude when considered in the light of the experience gained.

Flies, present in large numbers on the 1942 plots, were almost absent on the Clark Field. Honey bees, however, were plentiful during the treating period, especially on those plots with the highest concentrations of suspension. Ergot beetles (*Acylomus ergoti* Casey) were first seen in block I on June 20 and continued to be present throughout the field while the sclerotia developed. At no time were they as plentiful as on the Soils Field where there was an average of one beetle per head. From the few observations made, no relationship between the insects and the number of infections was established. It is probably true that insects are not a very important factor in the spread of ergot if the rye blooms and ripens evenly over the whole field.

During the time that this field was being treated the weather was bright and warm with only a trace of rain on the first day and with average humidity. From the results of this experiment and observations of others, it is believed that the success of this method of inoculating rye is not dependent upon hot, humid weather which is usually associated with natural epiphytotics of ergot. Because rain washes the suspension off the heads, it is detrimental. If the droplets remain from day to day, there is a good chance that as the flowers bloom the stigmas will come in contact with the droplets of suspension on the outside of the heads.

The Soils Field

Two adjacent plots of Rosen rye, 15×25 ft., were planted in October, 1942. Next to these, two plots of spring rye, each 30×25 ft., were planted in April, 1943. One of the fall rye plots was sprayed, using a hand sprayer, with a 1:1 dilution of high quality spore suspension, at the rate of 150 gal. per acre, between 8 and 10 A.M. on June 12, 14, 15, 16, and 17. This was during the period of most rapid blooming. The spring rye plots, which were untreated, began to flower June 22 and continued to bloom for 6 days during the maximum honeydew formation on the fall rye.

Sixty-one per cent of the heads in the treated plot, 10 per cent in the untreated fall rye plot, and 28 per cent in the spring rye plots produced sclerotia. The strip of spring rye 6 ft. wide adjacent to the treated plot had 44 per cent of the heads infected while in the most distant part there were only 23 per cent. For the whole field there was an average of 2.0 sclerotia per infected head.

Other 1943 Fields

One field sprayed three times with a hand sprayer using different concentrations of suspension produced rather poor results. The treated plots ranged from 10 to 20 per cent of the heads infected with less than 1 per cent in the controls. These poor results were probably due to the cool, misty weather that prevailed during half of the blooming period rather than to the method of application. Under these conditions the suspension of spores was washed off and the flowers did not open normally.

Fairly good results were obtained from one field which was treated three times with the power sprayer between 10 and 12 A.M. one day when the rye was at the peak of its bloom. Thirty-four per cent of the treated heads became infected, with 5 per cent in the controls. A field of spring rye treated with the power sprayer gave rather poor results when the total number of applications is considered. Three applications each day on three separate days with a 1:7 dilution caused only 60 per cent infection. The controls had 13 per cent of the heads infected. The poor results were probably due to the fact that spring rye grows poorly in Michigan: the plants were small and the heads very poorly filled. Perhaps a large number of flowers were infected without subsequently producing sclerotia.

GENERAL DISCUSSION

By weighing a number of medium sized sclerotia it was calculated that there are approximately 5,000 sclerotia per pound. On the basis of 1,200,000 heads of rye per acre the approximate yield of ergot in lbs. per acre can be computed by multiplying 1,200,000 by the percentage of heads infected, multiplying by the average number of ergots per head and dividing by 5,000. The figures thus obtained are likely to be misleading from a commercial point of view because of losses in harvesting. It was felt that acre yield estimates would be unreliable until later work with larger plots would actually show that a certain number of pounds of ergot could be harvested from large areas.

The problem of weather in relation to this method of inoculating rye with ergot has not been solved by the experiments presented, nevertheless two questions appear to be partially answered. Rain is definitely detrimental because it washes off the sticky droplets of suspension so they fail to come in contact with the pistils of later opening flowers. This was observed in 1942 and in 1943. Bright, clear weather appears to be favorable. Three of the four days during the treatment period on the Clark Field were bright; the first day had a trace of rain, but there was enough sunshine to dry it rapidly. Further research will be necessary to secure definite answers to the many questions about the relationship of weather of this method of inoculating with ergot.

It is obvious from information secured in the Clark Field that ergot does not spread far in a field of rye which blooms evenly during bright weather. The control strips which were adjacent to treated strips had a small percentage of infection, most of which could have been caused by blowing of the spray at the time of treatment. Probably the best evidence of failure to spread was obtained by examining the field of which these plots were a part. Only by careful searching could a sclerotium be found even within ten feet of the treated areas. Many authors have made note of this, but it bears repeating, because it is one of the first questions that comes up when one thinks of producing ergot artificially. It is obvious from the results of the Soils Field that if later blooming rye is nearby there may be considerable spread. This will undoubtedly also be true of grasses that are susceptible to the biologic races of ergot which attack rye.

The best time for the application of the spore suspension in relation to the blooming of the rye remains to be accurately determined. Rye flowers bloom in recurring intervals over a whole field. For 15 minutes a large percentage of the heads have from 1 to 5 flowers open. These close rapidly and for about 45 minutes almost no flowers in the field are open; then the blooming cycle begins again. The cycles begin about the time the sun strikes the field and continue all morning. The number of flowers that open in each cycle diminishes through the early afternoon so that by late afternoon blooming almost ceases. Cloudy weather and temperature changes influence the rhythm of bloom. These observations have not been carefully checked and

are of a tentative nature. However, based on them, it was concluded that the best time to spray is between 7 and 11 A.M.

A number of factors need further research before it can be said that the full capacity of this method to produce ergot is reached. Some of these are: (1) the most efficient method of applying the spore suspension, (2) the strength of the spore suspension, (3) the strength of the sugar solution used for the suspension, (4) the time of application, (5) the rate of application, (6) the number of applications, (7) the best age of culture to harvest for spores, (8) the best strain of the fungus, and (9) whether or not all applications should be made on one day or on different days. Add to these some factors which are general to this problem—(1) the variety of rye, (2) the site of the field, (3) the geographical location of the field, (4) machinery for harvesting the sclerotia—and one can see, in the light of the results already secured, that the possibilities of producing ergot artificially are good if the optima for these variables are determined.

SUMMARY

A method is described for the preparation of an ergot spore suspension which can be kept in cold storage for weeks and probably months, then diluted and used to inoculate rye plants in the field by spraying on the plants at blooming time. The suspension consisted of beaten and screened cultures to which was added an equal weight of beet sugar. A machine for the application of the spores is described. The results indicate that this is a good and relatively simple method of inoculating rye with ergot.

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THE EFFECT OF BRIEF TEMPERATURE TREATMENTS ON GERMINATION OF UREDIOSPORES OF PHRAGMIDIUM MUCRONATUM (FR.) SCHLECHT.[1]

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In the course of studies on the rate of germination of urediospores of *Phragmidium mucronatum* (Fr.) Schlecht., cause of the common leaf rust of cultivated roses, it was found that germination at low temperatures was greatly increased by exposure of the spores to room temperature during the period needed to make microscopic counts. In view of the fact that this exposure never exceeded 10 minutes, the phenomenon was judged of sufficient interest to warrant further investigation.

MATERIALS AND METHODS

A single-urediospore line of *Phragmidium mucronatum* was cultured on the Hybrid Tea variety Briarcliff. This clone was obtained from the rose variety Christopher Stone, grown in California.[2] Mature urediospores were brushed from infected leaflets into a beaker and transferred to a miniature "puff-duster" made of glass tubing. The spores were blown onto the surface of 5.0 cc. of 2 per cent water agar contained in a Syracuse watch glass. The watch glass and agar were held at the planned temperature of incubation for at least 8 hours before the spores were sown. Sown plates were stacked to prevent drying of the agar, and were incubated at the desired temperature.

Germination was counted microscopically in areas of the agar plate containing 75–150 spores per sq. mm. In each replicate 250 spores were counted for each observation; 2 or 4 replicate plates were sown for each treatment. The significance of differences between treatments and the agreement of replicates were assessed by the Chi-square method.[3]

Previous experiments had shown 18° C. to be the optimum temperature for urediospore germination, and all check lots were held at this temperature. The limits of urediospore germination are 6° and 28° C.; germination in appreciable percentage occurs only over the range 9–25° C. Temperature chambers used were equipped with thermoregulators accurate to ± 1° C.

RESULTS

In the first group of experiments spores were sown on agar at a low temperature, 6°, 8°, or 9° C. At stated intervals over a period of 36 hours 2 or

[1] Excerpt from a thesis presented April, 1944, to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer is indebted to Dr. H. Earl Thomas, Dept. of Plant Pathology, University of California, Berkeley, Cal., for the original supply of rusted leaves.

[3] McCallan, S. E. A. and F. Wilcoxon. The precision of spore germination tests. Boyce Thompson Inst. Contrib. **4**: 233–243. 1932.

4 of the plates were placed for a short period (10 minutes) in an incubator at 27° C., then returned to the original low temperature. Thus, at the end of an experiment there were available for comparison plates which had been warmed at each of several different time periods. Germination on these various plates was then determined and compared with that of spores on plates held uninterruptedly at the low temperature.

TABLE 1.—*The effect of a 10-minute exposure to 27° on spores germinating at 6°, 8°, or 9° C. Watch glasses containing germinating spores on agar were taken from the low temperature chamber at various intervals during the germination process and were held 10 minutes at 27° C., then were returned to the low temperature chamber. Germination on the treated plates was counted 24 hours after the time of exposure; germination of the control was counted at 48 hours from the time of sowing*

Experiment	Base temperature	Exposed at (hours from sowing)	Total counted	Per cent germination[a]	Per cent increase[b] over control
1	9° C.	(control)	500	68.2	0.0
		4 hr.	500	94.0	37.8**
		8	500	92.4**	35.5**
		12	500	87.2	27.9**
		24	500	79.4	16.4**
		36	500	65.6	−3.8
		48	500	70.6	3.5
2	8° C.	(control)	1000	28.3	0.0
		2 hr.	1000	52.3	84.8**
		4	1000	59.3	109.5**
		6	1000	63.8	125.4**
		8	1000	50.0	76.7**
		12	1000	44.9**	58.7**
		24	1000	33.8	19.4**
		36	1000	30.5	7.8
3	8° C.	(control)	500	22.8	0.0
		2 hr.	500	65.8	188.6**
		4	500	64.6	183.3**
		6	500	65.8	188.6**
		8	500	61.6	170.2**
		12	500	46.4	103.5**
		24	500	17.4	−23.7
		36	500	19.2	−15.8
4	6° C.	(control)	1000	5.7	0.0
		2 hr.	500	29.2	412.3**
		4	500	30.8	440.4**
		6	500	30.0**	526.3**
		8	500	17.6	208.8**
		12	500	9.4	64.9**
		24	1000	5.3	−7.0
		36	500	5.2	−8.7

[a] Double asterisk in this column indicates significant (99:1) difference among replicates.

[b] Double asterisk in last column indicates significant (99:1) increase over control.

The data of 4 experiments are in table 1. The time of exposure (Column 3) was measured from the moment of sowing. Thus, for example, the second line in table 1 represents the germination of 2 replicate spore samples held 4 hours at 9°, then 10 minutes at 27°, and finally 24 hours at 9° C. The control in each case was the germination of spores held continuously at the low

temperature. Total viability was determined by germinating a sample of spores at 18° C., and in no experiment was germination at this temperature lower than 90 per cent.

The figures of the last column of table 1 express the magnitude of the increase in germination caused by the exposure to 27° C. In all cases the effect of the treatment was significant at exposure times up to 12 hours from the time of sowing, and in two experiments (1 and 2) significant increases in germination resulted from exposure at 24 hours. The effect was greatest during the first 8 hours, declining thereafter and disappearing at 36 hours (24 hours in Experiment 3 and 4). In other words, the spores cease being receptive to the stimulus of the warming after 12–24 hours, and the maximum effect of such treatment is obtained when the exposure is within 8 hours of sowing the spores. In this connection, it may be noted that, with spores held continuously at 9° C., germination is first evident 4 hours after sowing, and that no increase in germination occurs beyond 10 hours from sowing of the spores.

When the period of exposure to 27° C. was reduced to 5 minutes, increases of up to 90 per cent were obtained; in this case the base temperature was 9° C.

A population of senescent spores responded in the same way as the fresh spores. Germination at 9° C. was 8.1 per cent in this case; exposure to 27° C. for 10 minutes at 6 hours from sowing raised the germination to 36.4 per cent. A sample from this population proved to be 83.5 per cent germinable at 18° C.

A further experiment, using a base temperature of 3° C. and fresh spores, was performed. In this case exposure to 27° C. for 10 minutes failed to bring about any germination; as noted previously, no germination occurs at 3° C.

One possible explanation of these results is the occurrence of condensation when the cold plates are set in a warm chamber. To examine this possibility, in one experiment sterile tap water was substituted for agar as the germination medium. The results were in all respects similar to those on agar: increases of up to 70 per cent were obtained by a 10-minute exposure to 27° C. of spores germinating at 9° C.

A second possible factor is exposure of the spores to light during the transfer from one chamber to another. To test this, two experiments were made to investigate the effects of warming of plates wrapped in black paper. Although the wrapping in paper reduced germination in all plates, the response to warming was proportionately the same as in unwrapped plates. Thus, in one case exposure of wrapped plates for 10 minutes to 27° C. (incubation temperature 9° C.) 4 hours after sowing increased germination from 8.0 per cent to 31.2 per cent.

No measurements of the temperature at the agar surface were made. "Exposure to 27° C. for 10 minutes" does not, therefore, imply that the treated spores reached or were maintained at this temperature. The Syra-

cuse watch glasses bearing the spores were not stacked during the exposure to the high temperature, but were spread out singly. The spore on the agar surface was thus presumably separated by only a thin layer of water from air at 27° C. Undoubtedly the spores rose in temperature, but the precise amount of increase was not determined.

A related phenomenon was investigated in the experiment summarized in table 2. Agar plates sown with urediospores were held for one hour at 18° C., then for 23 hours at various temperatures known to be non-optimal for germination (3°, 6°, 9°, 24°, 27°, and 30° C.). No germination occurs during the first hour of incubation at 18° C. After 23 hours at the various temperatures germination of each lot was determined and compared with that of control spore samples incubated the entire 24 hours at the same tem-

TABLE 2.—*The effect of a preliminary one-hour exposure of germinating urediospores to 18° C. on subsequent germination at various unfavorable temperatures. Spores were sown on 2 per cent water agar. Treated spores held one hour at 18° C. and 23 hours at an unfavorable temperature were compared with control spores held 24 hours at the unfavorable temperature. Each per cent germination, except as noted, was based on a count of 250 spores in each of 4 replicates*

Temperature, °C.	Per cent germination		Per cent increase[b] over control
	Control	Treated	
3	0.0	1.7	.....
6	0.8	6.6	725.0**
9	6.5	17.1	163.1**
18	96.6	.....	.....
24	77.6	90.0[a]	16.0**
27	5.0	20.4[a]	308.0**
30	0.0	1.3	.....

[a] Based on a count of 250 spores in each of 2 replicates.
[b] Double asterisk indicates significant (99:1) increase over control.

perature. In table 2 each lot of treated spores is compared with spores held for 24 hours, with no preliminary incubation at 18° C., at the unfavorable temperature.

From the data of table 2, it is evident that the one-hour exposure to 18° C. had a significant positive effect, even though at the end of the one-hour period no germination had been visibly initiated.

Consideration of the data of table 2 suggests a point of some interest in relation to methods of spore germination. In temperature trials of germination it would seem desirable to insure that the germination medium is at the control temperature before the spores are sown, rather than to sow the medium at laboratory temperature and only then to place the vessels at the experimental temperature.

Evidence on this methodological point was obtained and is presented in table 3. A comparison was made between agar plates sown at 18° C. and placed immediately at the low experimental temperature (7° or 9° C.), and similar plates held 24 hours at the low temperature before sowing. The latter are designated "precooled."

In temperature studies of spore germination the medium should be brought to the desired temperature before the spores are sown. Some investigators[4] have taken this precaution, others probably have not.

DISCUSSION AND SUMMARY

Large increases in germination of urediospores of *Phragmidium mucronatum* at 6–9° C. were brought about by brief warming of the germinating spores in an incubator at 27° C. The increases were greatest when exposure to the high temperature was made during the first 8 hours after sowing of the spores; exposure at periods of more than 24 hours after sowing had no effect.

The actual temperature of the agar during the warming in the high temperature chamber was not determined; it is therefore impossible categori-

TABLE 3.—*The effect on urediospore germination at low temperatures of a preliminary cooling of the germination medium to the control temperature. Germination on precooled agar was compared with that on agar sown at room temperature (20° C.) and then placed at the low temperature. Germination at 18° C. was 93.9 per cent. Each per cent germination was based on a count of 250 spores in each of 4 replicates*

Temperature	Treatment	Per cent germination[a]
7° C.	Precooled	6.1
	Not precooled	24.4**
9° C.	Precooled	50.1
	Not precooled	79.0**

[a] No significant differences among replicates. Double asterisk indicates significant (99:1) increase over germination on the corresponding "precooled" agar.

cally to state that any particular temperature is decisive. The results suggest that there may exist some reaction—or reactions—in the germination process which is limiting at 6–9° C. and which can be completed in a relatively short time at a higher temperature.

The germination of urediospores at temperatures too high or too low for optimum germination was significantly increased by a preliminary one-hour incubation at 18° C., the optimum temperature for germination in this species. Again, the speculation that a temperature-sensitive key reaction may be completed during the period at 18° C. is suggested by these data.

From the standpoint of spore germination methods, these results indicate that extreme care is necessary, in temperature tests of germination, to insure that the germinating spores are always at the desired temperature. In practice this requires a preliminary incubation of the germination medium at the desired temperature. Further, in studies on the rate of germination, the data show that either counts should be made at the control temperature or the samples counted at a higher temperature should be discarded.

[4] Straib, W. Physiologische Untersuchungen über *Puccinia glumarum*. Centr. Bakt. II, **102**: 154–188. 1940.

In nature a short period of favorable temperature may result in spore germination on the leaf even though temperature is generally unfavorable. This consideration suggests caution in applying the results of laboratory experiments to field problems.

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PHYTOPATHOLOGICAL NOTES

Effect of Vernalization on the Development of Stripe in Barley.[1]—Classification studies of North American barley varieties required the growing of a winter barley nursery at the experiment station in Aberdeen, Idaho, in 1944. As this nursery was to be planted in the spring, two series of seed were prepared and one of them was vernalized. The seed for the planting came from a classification nursery grown at Raleigh, North Carolina, in 1943. The seed to be vernalized was placed on blotters in Petri dishes in a refrigerator at the Plant Industry Station in Beltsville, Maryland, on February 23, 1944, where the vernalization was under the supervision of Dr. G. A. Wiebe. Sufficient water was added to keep the barley moist. The

TABLE 1.—*Percentage of striped plants or culms developing from vernalized and nonvernalized seed lots*

Barley strains	C.I.[a] No.	Estimated percentage of striped plants[b]				Percentage of striped culms at harvest, July 29	
		June 16		June 28			
		Non-vernal-ized	Vernal-ized	Non-vernal-ized	Vernal-ized	Non-vernal-ized	Vernal-ized
Hooded:							
Brugh 76	6477	trace	2	5	45	9	62
Huga	6998	0	5	0	20	1	23
Tucker	7039	0	5	trace	30	4	48
Missouri Early Beardless	6051	trace	10	trace	30	6	42
Hooded 16	6574	0	5	trace	10	1	15
Iredell	6571	2	15	10	50	9	60
Rough Awned:							
Wintex	6127	trace	20	5	50	6	45

[a] C.I. refers to accession number of the Division of Cereal Crops and Diseases.
[b] Seed not space planted, therefore exact counts impossible.

moisture content was kept constant during a vernalization period of 38 days or until April 1. The temperature in the refrigerator was 33–34° F. from February 23 until March 15, and 31–32° F. from March 16 until April 1. At the end of the vernalization period some barley kernels had roots just breaking through the coleorhiza, others had roots from a few mm. to 2 cm. long. After April 1 the vernalized seed was dried at room temperature for 3 days and then mailed to Aberdeen, Idaho, where it was planted on April 8.

In the field, paired 10-foot rows of vernalized and nonvernalized seed of the same barley strain from the same seed source made direct comparisons possible. During the summer barley stripe (*Helminthosporium gramineum* Rabh.) was observed on a few of the winter barleys. At the beginning of June the vernalized rows of 7 barley strains had typical stripe infection

[1] Investigations supported in part by a grant from the Wisconsin Alumni Research Foundation.

while no stripe was noticeable on the nonvernalized rows of the same barley strains. Later in the season there was a definite difference in the amount of stripe on the vernalized and nonvernalized rows. The observations made during the growing season and the counts on the healthy and striped plants at harvest in the latter part of July are given in table 1.

The vernalization process favored the development of stripe. It is possible that the mycelium from diseased seeds infected the healthy seedlings. These results indicate that it might be possible to develop a technique for testing varietal resistance to barley stripe on the basis of a vernalization procedure.—EWERT ÅBERG, University of Wisconsin and Bureau of Plant Industry, Soils, and Agricultural Engineering, Plant Industry Station, Beltsville, Md.

Control of the Gall Disease of Gypsophila Caused by Phytomonas gypsophilae (Brown) Magrou.[1]—The gall disease on newly grafted *Gypsophila paniculata* caused by *Phytomonas gypsophilae* (Brown) Magrou[2] (*Bacterium gypsophilae* Brown[3]) was adequately described by Brown.[3, 4]

In the last 4 years several New Jersey growers of this plant have experienced increasing losses from the disease. Under New Jersey conditions, well-developed galls appear within 2 weeks after the plants are grafted. The galls appear mainly on the upper cut surface of the rootstocks in the region where the scion is inserted. They are soft and nodular and vary in diameter to 4 cm. As a result of the excessive growth of the galls, the scions fail to form a strong union with the rootstock and most of the plants die within a month. Where a gall develops along the inside slit in the root, the scion commonly is pushed completely away from the root, thus resulting in the death of the plant.

Control Measures. Brown[4] recommended dipping gypsophila roots to be used for understock for 1½ to 2 min. in a 1–1000 mecuric chloride solution in order to kill the gall bacteria that may be present on the surface. No injury to the roots was reported.

The same dip was tried by the author in June, 1942, and May, 1944, on several hundred newly grafted gypsophila plants but it caused considerable damage. A suspension of calomel made by dissolving 1½ oz. gum arabic in a gallon of water and adding 2 oz. calomel also caused injury. Apparently, in the confined areas of the frames in which newly grafted plants are placed, the mercury still adhering to the dipped plants volatizes in sufficient amounts to cause considerable injury.

In May, 1944, and at periodic intervals throughout the summer and early fall, the value of other materials was determined. As a result it has been

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Dept. of Plant Pathology.

[2] Bergey's Manual of Determinative Bacteriology. Fifth Edition, p. 210. Williams & Wilkins, Baltimore. 1939.

[3] Brown, Nellie A. Another gall-forming bacterium. Phytopath. 22: 924–925. 1932.

[4] Brown, Nellie A. A gall similar to crown gall produced on Gypsophila by a new bacterium. Jour. Agr. Res. [U. S.] 48: 1099–1112. 1934.

found that a 2-min. dip of newly grafted plants in a calcium hypochlorite solution controls the disease without injuring the plants. Ordinary calcium hypochlorite sold in grocery and drug stores as chloride of lime was used at concentrations ranging from 2 to 12 oz. of the powder per gallon of water. Concentrations from 2 to 6 oz. per gallon caused no plant injury and still controlled the disease. The 12-oz. per gallon rate injured the foliage and was discontinued in later tests.

The preparation of the calcium hypochlorite solution is simple. The day before the solution is to be used, dissolve the powder in water and pass the solution through filter paper.

Table 1 presents the data collected from three typical tests during 1944.

TABLE 1.—*Calcium hypochlorite dip to control gall disease of grafted gypsophila*

Date treated	Oz. chemical per gal. H_2O	Treated plants		Control plants	
		No.	Per cent healthy	No.	Per cent healthy
July 15	6	25	100.00	25	4
Aug. 25	6	125	99.92	125	25
	3	125	97.60	125	36
Sept. 16	4	125	99.00	250	50
	2	125	98.00	250	60

These data clearly indicate that the gall disease can be controlled by dipping newly grafted gypsophila plants for 2 to 3 min. in a calcium hypochlorite solution made by dissolving from 2 to 6 oz. of the powder in a gallon of water.

Among the materials tested and discarded because they caused plant injury or failed to control the disease were the 1–1000 mercuric chloride solution and calomel suspension mentioned; U. S. Rubber Company, Naugatuck Chemical Division Compound 604; and phenyl mercury salycilate dip, which is made by dissolving 1 part of the chemical in 400 parts of water.—P. P. PIRONE, New Jersey Agricultural Experiment Station, New Brunswick, N. J.